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VOLUME 43 SEPTEMBER, 1953 NUMBER 9

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

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News

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FORTY-FIFTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY MADISON, WISCONSIN, SEPTEMBER 7, 8, 9, 10, 1953

Origin of Peach Bacterial Spot Spring Cankers. ADAM, ANGELOS, H. W. ANDERSON AND DWIGHT POWELL. Time of infection of twigs in relation to spring canker development was determined by artificial inoculation by forced spray and needle puncture on 1-year-old disease free trees. Forced spray inoculations were on July 3, August 4, 8, September 8, 26 and October 18. Needle inoculations were on September 3, 18, October 5, 24. Isolations from the needle punctures were attempted at approximately weekly intervals from October 28 to April 3, 1953. Successful isolations were obtained from 70 per cent of September 3 inoculations, 43 per cent of September 18, 22 per cent of October 5 and 2.5 per cent of October 24 with none from control punctures. No successful isolations were obtained from October 24 inoculations after December 5. Isolations from individual punctures showed a higher survival in first and second punctures below tip than in 3 lower punctures. In plots inoculated by forced spray August 4, September 8, 26, and October 18, no leaf infection appeared in 1952 but abundant spring cankers and primary infection areas were found in 1953 with none on adjoining noninoculated trees. All trees inoculated by either method had spring cankers and areas of primary infection. The conclusion is that twig infection giving rise to spring cankers may occur at any time from the first of August to the middle of October.

Mist spraying less injurious than hydraulic spraying to sour cherries and apples. ADAMS, ROBERT E. Mist spray applications were compared directly with gun spray applications. In an experiment on Montmorency cherries in 1951, and repeated on the same trees in 1952, Copper Compound A was applied at 1, 2, 4, 6, and 8 times the concentration applied with the gun. The same amount of toxicant was applied to the trees in each treatment with the exception of the 6× mist spray, which was applied at ¾ the rate of the others. Soluble solids, fruit size, and tree trunk circumference determinations were made. In a similar experiment on McIntosh apples, wettable sulfur was applied at 1, 2, 4, and 8 times concentration. Formulations containing no adjuvant, 1/20-pt. Triton B-1956, ½-pt. and 1-pt. Veg Oil in 100 gal. were applied at each concentration. Visible foliage and fruit injury, pre-harvest drop, soluble solids in fruit, fruit size, and chlorophyll content of foliage were determined. Equal or superior disease control and less injury, except with the 8× mist spray on cherries, resulted from the mist spray than from the gun spray treatments. The results obtained indicate that mixtures containing different adjuvants differ both in the severity of injury caused and disease control obtained.

Laboratory studies of effectiveness of 3,5-dimethyltetrahydro-1,3,5,2-thiadiazine-2-thione against certain plant parasitic fungi and nematodes. ANDERSON, E. J. AND MARION OKIMOTO. The chemical, obtained as Crag Fungicide 974, completely inhibited growth of *Phytophthora cinnamomi*, *Fusarium moniliforme* and *Penicillium* sp. at 100 p.p.m. and of *Pythium arrhenomanes* at 10 p.p.m. in culture plates. Fumigant action was demonstrated in tests wherein fumes from 10 mg. of the moistened chemical or 400 mg. of the dry chemical killed *P. cinnamomi* in 250 ml. culture flasks. In these tests the original toxicity was maintained by the moistened chemical for 25 days. After 25 days toxicity decreased. In potted soil tomatoes grew normally 10 days after treatment at the rate of 100 lbs. per acre foot. Root rot due to *P. cinnamomi* was completely controlled in pot tests by treatment of the soil at the rate of 50 lbs. per acre foot. A mixed population of nematodes in potted soil was reduced from 620 to 20 per 100 gm. by treatment at the rate of 50 lbs. per acre foot and to 3 by the 100 lb. rate. Tomato indicator plants had over 500 galls in untreated rootknot infested soil as compared with one gall in soil treated at the 50 lb. rate and none at 100 lbs.

Physiologic races of the wilt Fusaria from cabbage, radish, and stock. ARMSTRONG, G. M. AND J. K. ARMSTRONG. In a previous paper, the cabbage and radish wilt Fusaria were shown to be races 1 and 2, respectively, of *F. conglutinans* Wr. An isolate of the stock wilt *Fusarium* from California and one from New York have also been included in further studies of the host relationships of the Fusaria causing wilts of the Cruciferae. Plants of various members of the cabbage tribe, radish, stock, garden cress, rock cress, wallflower and Aubrietia sp. were inoculated with each of the Fusaria; candy tuft and sweet alyssum with all but the isolate from New York. Evidence was obtained that the California stock wilt *Fusarium* is race 3. The N. Y. isolate differed from the California isolate in its pathogenicity to certain varieties of stock and other Cruciferae. It resembled the cabbage wilt *Fusarium* in its reaction to these hosts but differed from it in causing slight to no wilt of Early White Dutch turnip and Wisconsin All Season Resistant cabbage which are susceptible to the cabbage wilt *Fusarium*. The indications are that this is race 4. Southern Giant Curled mustard was highly susceptible to all races.

Caryophyllaceae susceptible to the carnation-wilt Fusarium. ARMSTRONG, J. K. AND G. M. ARMSTRONG. Three varieties of carnation, several varieties of sweet william, several species of garden pink, and *Lychnis chalcedonica* are susceptible to single-spore isolates of the carnation wilt *Fusarium*. The wilt *Fusarium* from sweet william, which was described as a new form, was not available for comparison. Since sweet william is a common host, these Fusaria are either the same fungus or physiologic races of the carnation wilt *Fusarium*. The carnation fungus was used to inoculate 29 species and varieties of wilt susceptible plants which were not Caryophyllaceae, and 24 different wilt Fusaria which attack some of these plants were used to inoculate 1 variety each of carnation and sweet william. Cross-inoculations failed to reveal a common host since there was no wilting of any plant.

Vapor action of certain mercury seed treatment materials. ARNY, D. C. AND CURT LEBEN. The vapor action of 4 seed treatment fungicides was assessed by placing the fungicide and oat seed infested with *Helminthosporium victoriae* in a closed container for various exposure periods. There was no direct contact between seed and fungicide. The amount of fungicide vapor taken up by the seed was measured by the Glomerella agar diffusion assay and was indicated also in greenhouse tests by the incidence of the Helminthosporium seedling blight. According to the bio-assay, Panogen was highly active across the air space, Ceresan M was slightly active, while Setrete and Agrox were relatively inactive. Seed exposed to Panogen and Ceresan M showed some activity after the hulls were removed, indicating that the vapor of these materials penetrated beneath the hulls. Panogen gave good control of seedling blight after 1 day exposure, Ceresan M only after more than 8 days, while Setrete and Agrox gave poor control even after 32 days' exposure. With a fixed amount of Panogen in the closed container, the amount of vapor taken up by the seed in the surface layer was inversely proportional to the amount of seed in the container, and the vapor penetrated less than 2.5 cm.

Effect of soil fumigation on growth and yield of Valencia orange trees. BAINES, R. C., AND J. P. MARTIN. Preplanting soil fumigation treatments on Romona sandy loam soil previously cropped 25 years to oranges increased growth and yield of young Valencia orange trees. Chemicals were injected at 10 or 20 in. depths at 20-in. spacing. Plots 24 × 40 feet were replicated 4 times. The treatments rated on trunk-cross section area of 5-year-old Valencia orange trees were 700 and 1400 lbs. D-D (dichloropropene-dichloropropane

mixture)/A., 315 lbs. ethylene dibromide, 1100 lbs. chloropicrin, 2000 lbs. carbon disulfide, 630 lbs. ethylene dibromide, 550 lbs. chloropicrin, 1000 lbs. carbon disulfide, and untreated, in descending order. Five-year-old trees on the fumigated soil averaged 10–28 lbs. of fruit per tree and on untreated soil, 3 lbs. All D-D and ethylene dibromide treatments eradicated the citrus nematode in the 0–5 ft. depth sampled, while approximately 2 and 10 per cent of the nematodes survived the chloropicrin and carbon disulfide treatments, respectively. In treatments applied in 1951, 500 and 700 lbs. of D-D/A. eradicated the citrus nematode and increased tree growth similarly. The deep injection of the chemicals did not result in increased control of the nematode nor of improved tree growth and yield.

Recovery of viable spores of Endoconidiophora fagacearum from excrement of insects used in disease transmission studies. BART, GEORGE J., AND CHARLES L. GRISWOLD. It was observed that *Drosophila melanogaster* Meig. was attracted to and fed upon sporulating mats of *Endoconidiophora fagacearum* and also the bleeding wounds of healthy oak seedlings in the laboratory. This insect was also taken in traps baited with sporulating mats of the pathogen in a woodland area containing diseased trees. These observations suggested the possibility of *D. melanogaster* being a vector of the oak wilt fungus. Excrement produced by adult flies which fed upon the fungus was found to contain numerous endospores. A suspension made from the excrement was smeared upon agar plates resulting in the development of several typical oak wilt fungus colonies. Virulence was not lessened by passage of the pathogen through the intestinal tract of the files as disease symptoms subsequently appeared in healthy oak seedlings inoculated with spores from these colonies. Certain unidentified species of Nitidulidae were found to be similarly attracted to fungus mats and wounds in the field. Excrement from these insects contained large numbers of endoconidia. The viability of these spores has not yet been determined.

Dieback and canker of camellias. BAXTER, L. W., AND A. G. PLAKIDAS. Dieback is a widespread and often destructive disease of camellias throughout the South. It is characterized by the formation of cankers and the dying back of twigs and larger shoots. There has been considerable disagreement among previous investigators regarding the cause of the disease and factors favoring its development. The present investigation, carried on over a 3-year period, has shown: 1) The cause of dieback is *Glomerella cingulata*; typical symptoms resulted from inoculations with pure cultures of the fungus on 20 varieties of *C. japonica* and 2 of *C. sasanqua*. 2) Wounds are necessary for infection; in nature the scars left by naturally abscissing leaves are the most common points of ingress, and the period of greatest onset of dieback coincides with the period of shedding of the old leaves in the spring. 3) Infection, resulting in typical symptoms, was repeatedly secured by applying conidia to the scars of naturally abscissing leaves, of leaves induced to absciss by deblading, and of leaves removed mechanically. 4) The imperfect stage of the fungus is a *Colletotrichum* (setae have been found) and not a *Gloeosporium* as previously reported.

The prevention of damping-off of coniferous seedlings by pelleting seed. BERBEE, JOHN G., FLORA BERBEE, AND W. H. BRENER. Twenty-eight fungicides were tested during the past 3 years as soil and seed treatments to prevent damping-off of coniferous seedlings incited by *Pythium irregulare* and *Rhizoctonia solani*. These diseases, particularly *Rhizoctonia* damping-off, have continued severely in red pine seedlings in Wisconsin. A single spring soil application of 75 per cent tetramethylthiuram disulfide or of a 30 per cent mixture of the sodium salts of dimethyldithiocarbamic acid and 2-mercaptobenzothiazole sprayed at the respective rates of 0.5 and 4.0 gm. per sq. ft. controlled post-emergence Rhizoctonia damping-off and reduced weeds in fall or spring sown seedbeds. Pre-emergence losses occurred and both treatments attracted mice. Pelleting seed with 50 per cent tetramethylthiuram disulfide, using 4 per cent methyl cellulose as a binder, was more effective than treating soil. Concentrations up to 4 lb. of fungicide to 1 lb. of seed caused no injury in fall sown seedbeds. Pellets prepared in a pharmaceutical pill coater by mixing 3 oz. of methyl cellulose, 8 oz. of fungicide, and 1 lb. of seed controlled both pre- and post-emergence infections. In spring seedlings, a mixture of 1 oz., 2 oz. and 1 lb., respectively, provided adequate protection.

Studies on Verticillium albo-atrum isolated from pepper and eggplant. BERAHA, LOUIS, AND M. B. LINN. Isolates of *Verticillium albo-atrum* from peppers and eggplants from several states were found to be pathogenically distinct. Pepper isolates from California, Illinois, and Russia (all micro-sclerotial types) were pathogenic to 12 varieties of pepper and 3 varieties of eggplant, while eggplant isolates from Illinois, New York, and New Mexico (latter only dark mycelial type) were pathogenic to eggplant but not to pepper. The pepper isolates from Illinois and California were extremely pathogenic to pepper and eggplant while the isolate originally from Russia was less damaging. Similarly, the New Mexico and Illinois isolates from eggplants were extremely virulent while the New York isolate gave a lower disease index. Differences were also found when these isolates were used in cross inoculations with other suscepts. Studies with the 6 isolates on 3 natural media and 1 synthetic medium demonstrated cultural differences among them. Variation in susceptibility ranging from extremely susceptible to immune within pepper varieties suggest the possibility of selecton and hybridization for development of resistant varieties.

Loss of vector transmissibility by viruses normally insect transmitted. BLACK, L. M. An isolate of *Aureogenus vastans* was maintained in plants without insect transmission for 16½ years and likewise 2 isolates of *A. vastans* var. *agalliae* for 12 years. Four hundred and fifty-four vectors fed on *Trifolium incarnatum* infected with these isolates failed to transmit them whereas 433 control insects that had fed on *T. incarnatum* infected with fresh isolates from the field produced 102 infections when tested in colonies. These results are interpreted to mean that during maintenance of the viruses in plants without insect transmission, their property of transmissibility by specific vectors was lost through mutation and that, under these conditions, the mutated forms possessed an advantage in energy relations. It seems probable that viruses that have lost vector transmissibility will be found in plants propagated vegetatively for long periods as is the case with many cultivated plants. Paracrinkle virus may have been spread throughout the King Edward clone by insects and may have subsequently lost its vector transmissibility. A similar explanation may account for Orlando and Silberschmidt's failure to transmit infectious chlorosis of *Abutilon thompsonii* by *Bemisia tabaci*. Virus strains, normally transmitted only by vectors, that have lost vector transmissibility may be safe subjects for investigation outside their current range.

Effect of nutritive foliage sprays on physiology and development of Fusarium wilt of tomato. BLOOM, JAMES R. Bonny Best tomato plants growing in Hoagland's balanced solution in quartz sand continuous drip culture were given supplementary nutrient by spraying the foliage with various concentrations of urea, sodium glycerophosphate, potassium formate, KCl, $MgSO_4$, $CaCl_2$, $(NH_4)_2SO_4$, $NaSO_4$, NaH_2PO_4, NaCl, $Ca(NO_3)_2$, and $MgCl_2$ before and after inoculation with *Fusarium oxysporum* f. *lycopersici* (Sacc.) (S. & H.). Sucrose was used alone and was added to each other spray to prevent foliage burning. All the materials caused increase in disease with increased nutrient concentrations when applied after inoculation, except potassium formate and $MgSO_4$ which decreased disease with increased concentrations. Some nutrients gave opposite results when applied before, as compared with after, inoculation. High concentrations of potassium on foliage increased disease while high potassium supplied through roots decreased disease. Transpiration was decreased by nutrients which increased disease and was increased by $MgSO_4$ and potassium formate which decreased disease. No correlation has been found between respiration and disease as influenced

by nutrition. High potassium on foliage apparently retarded the action of amylase in the conversion of starch to sugar. Stem tissue contained higher percentages of potassium than leaf tissue, while leaf tissue contained the higher percentages of magnesium.

Hessian fly, Phytophaga destructor (Say), in relation to crown and foot rot of wheat. BOOSALIS, MICHAEL G. AND HENRY JEDLINSKI. Experiments were made to corroborate field observations indicating a relation between Hessian fly and crown and foot rot of wheat. The insect susceptible variety Cheyenne and the resistant Cheyenne × Ponca were infested in the greenhouse before hardening; in a parallel series infestation was accomplished after hardening. Each series contained 6 treatments, 60 plants per treatment and was replicated 4 times. Infested plants were grown in 1) sterilized field soil, 2) unsterilized soil, 3) sterilized sand. Treatments 4, 5, and 6 were similar to 1, 2, and 3 respectively except that the plants were insect-free. Observations during heading stage revealed that about 60 per cent of Cheyenne infested before and after hardening and grown in unsterilized soil was severely infected with crown and foot rot. A less severe rot developed on 25 per cent of infested Cheyenne plants grown in sterilized soil. No appreciable rot was discerned on Cheyenne from the other 4 treatments nor on Cheyenne × Ponca with any of the 6 treatments. None of the isolates per se of *Helminthosporium* spp. and *Fusarium spp.* from diseased plants were pathogenic to wheat. Preliminary results from experiments in which the sterilized soil was maintained aseptically substantiate the above findings.

Jelly end rot of potatoes. BOOTHROYD, C. W., AND R. C. CETAS. Symptoms of a disease resembling jelly end rot were observed in the 1949 and 1952 potato crops on Long Island, New York. The stem end of the tubers rotted. The inner tissue was devoid of starch, high in sugars, and had a wet jelly-like consistency. Failure to find 1 or more microorganisms consistently associated with the rotted tissue suggested a disease of non-pathogenic origin. The average of the minimum-maximum temperatures for the growth period of the potato (April through September) was generally higher for those years in which jelly end rot occurred. Rainfall data for the same period revealed insufficient rainfall for normal potato growth in mid-season followed by adequate to abundant rainfall thereafter. It is believed that high temperature and a low water supply followed by a high one from mid-season on may be important in the incidence of jelly end rot. Data taken at harvest in a potato variety plot revealed a variation in percentage of jelly end rot from 1.7 to 24.3 per cent. Kennebec and Katahdin showed the least jelly end rot, and Cherokee and Canso the most. Seedlings in the same plot showed the same range of susceptibility.

New approach to the breeding of wheat varieties resistant to Puccinia graminis tritici. BORLAUG, NORMAN E. If a commercial wheat variety is to be developed which will have a possibility of remaining rust-resistant indefinitely, this variety must be constituted so that its resistance can be modified to meet changing relative prevalence of different races of *Puccinia graminis tritici.* It is proposed to produce such a "composite variety" by a modification of conventional backcross methods. The variety when distributed commercially, will be a mixture of a number of phenotypically similar lines, which are genotypically different for resistance. The "backcross lines" which are eventually employed in the "composite variety" are developed by crossing a commercial variety to a number of varieties carrying different types of resistance. Each single cross is backcrossed several times to the commercial variety. When the "composite variety" is ready for release, the several lines are multiplied separately, then mixed mechanically to form the variety for release to seed growers. With changes in races 1 or several of the lines in the "composite variety" can be removed or replaced. As many lines can be developed and held in reserve as there are types of resistance. Adequate race surveys, storage of spores by lyophylization technique, and adequate greenhouse testing of each line, are integral parts of this method. Currently we are attempting to improve 5 commercial varieties by this method.

A quick method of determining virus transmission through cherry seeds. BOYLE, JOHN S. Fruits from 10 Montmorency (9 yellows and 1 necrotic ring spot) and 3 St. Medard (2 yellow and 1 no symptoms) were collected in July, 1952, and 250 cleaned pits from each tree immediately stratified in wet peat moss at 35–40° F. In January, 1953, the flats were moved to a greenhouse maintained at approximately 60° F. After germination, young symptomless, cherry seedlings in the 2-leaf stage were selected at random from each seed source ground with 3–5 ml. of tap water and the extract mechanically inoculated to 4 National Pickling cucumber seedlings using the carborundum technique. A virus was transmitted to cucumber from 25 of 76 Montmorency seedlings and 8 of 15 St. Medard seedlings. Infection occurred on cucumber from all 13 seed sources. The highest percentage of transmission from any Montmorency seed lot was 50 per cent. There was 60 per cent transmission in one St. Medard lot. Forty inoculations (4 Montmorency seedlings from each of the 10 seed lots) to cucumber using older cherry seedlings in the 4 leaf stage that were hardening resulted in transmission in only 2 cases. These data indicate that cucumber may be used to determine the presence of virus in Prunus seed and show seed transmission in higher percentages than previously realized.

The combination of zone electrophoresis and density gradient centrifugation in the purification of some unstable plant viruses. BRAKKE, MYRON K. Concentrates prepared by 1 cycle of differential centrifugation followed by a cycle of density gradient centrifugation were subjected to zone electrophoresis. *Nicotiana rustica* was used as a source of *Aureogenus vastans* with 0.1 M pH 5.6 acetate buffer being used for electrophoresis in potato starch columns. The virus was located after electrophoresis by density gradient centrifugation of eluates of segments of starch columns. The zones obtained by density gradient centrifugation had previously been correlated with infectivity. *Aureogenus magnivena* was obtained from sweet clover stem tumors or viruliferous *Agallia constricta* and electrophoresis carried out by the same technique except that 0.02 M pH 6.0 phosphate buffer was used. There appeared to be a small amount of adsorption of these 2 viruses on the starch but much more adsorption of the normal plant constituents. *Lethum australiense* was obtained from *N. rustica* and electrophoresis carried out in a sucrose density gradient tube containing 0.01 M neutral phosphate buffer. The progress of the electrophoresis in the gradient tube could be followed by observing with the naked eye the light scattered by the particles, making it unnecessary to locate the virus by density gradient centrifugation.

Studies on the effect of Chalara fagacearum on oak wood and its effect on rates of rotting by associated wood-rotting fungi. BRANDT, WILLIAM H. In studies using small blocks of various species of oak, *Chalara* failed to weaken oak under the same conditions which permitted associated wood-rotting fungi to decrease the static-bending strength of the blocks more than 90 per cent, in some cases. Considering this and the fact that other workers have shown slight ability on the part of *Chalara* to utilize cellulose, it would seem that *Chalara* alone cannot damage oak timber. No significant difference at either the 5 per cent or 1 per cent level was found between the average static-bending strengths of groups of oak blocks subjected to an associated wood-rotting fungus alone and groups treated in various ways with *Chalara* or its metabolic products before being subjected to the associated fungus. Although there were a few indications that *Chalara* might enhance the rate of deterioration of oak wood by wood-rotting fungi, this has not as yet been demonstrable in the laboratory. A survey revealed several fungi commonly associated with *Chalara* which inhibited *Chalara* growing on agar.

The relationship of fungi to shell bark of lemon trees. CALAVAN, E. C. *Diaporthe citri, Diplodia natalensis, Botrytis cinerea,* and *Botryosphaeria ribis* in wound inoculations

have each been found capable of inducing and enlarging shell-bark lesions on susceptible Eureka lemon trunks. Typical shell bark was induced only when inoculations were made into mature bark of trees already showing indications of shell bark development. Naturally occurring shell-bark lesions usually contain no fungi at first, but are soon invaded by one or more species. The role of fungi in most shell-bark lesions is evidently that of invaders of dead or dying tissues. Lemon bark often reacts to these invaders by gum formation, additional necrosis, and phellogen development. The resistance of lemon trees to shell bark depends partly upon the presence of a strong wound-healing potential in the outer bark. In resistant bark, healing occurs through and beneath the cortex and delimits injuries, but in highly susceptible bark it proceeds only in tissues beneath the cortex and results eventually in the tangential separation of outer and inner bark layers.

Corticium galactinum and white pine needle blight. CAMPANA, RICHARD J. The reported association of *Corticium galactinum* with white pine needle blight has led to speculation that this fungus may be primarily responsible for the disease by destroying feeding rootlets. To test the virulence of the fungus as a pathogen on roots of white pine, the roots of 288 4- and 6-year old seedlings potted in coarse or loamy sand were inoculated in the greenhouse. Inocula consisted of 9 isolates of the fungus; there was 1 control seedling for each isolate used. From data on needle length and needle color, symptom condition indices were calculated for each seedling and an analysis of variance was made for the effect of inoculation. The incidence of needle blight symptoms was greater on the controls. In an examination of root systems of inoculated seedlings there was no evidence that the fungus had invaded the roots. Attempts to isolate the fungus from inoculated roots were unsuccessful. In a survey of root fungi isolated from roots of 160 adjacent, diseased and healthy trees *C. galactinum* was never isolated. No evidence was obtained from this study to suggest *C. galactinum* as a cause of white pine needle blight.

Correlation of seedling and adult plant reaction to stem rust of wheat. CAMPOS, T., A., J. W. GIBLER, AND N. E. BORLAUG. For many years it has been known that some varieties of wheat are susceptible in the seedling stage to a given race of stem rust but are resistant in the adult stage. Until recently, it was generally accepted that varieties resistant in the seedling stage are always resistant to the same race in the adult plant stage. More recent studies have shown that in crosses involving the variety Mentana many lines which are resistant in the seedling stage are susceptible in the adult stage, whereas other lines in the same cross remain resistant throughout their life. Recent experiments involving both seedling and adult reaction of 1500 varieties and lines of wheat to races 15B and 49 establish that the phenomona of seedling resistance and adult plant susceptibility are not unique to crosses involving Mentana. More than 200 crosses were studied during the winter of 1952-53 and 34 of these exhibited the phenomena, and many lines showing the change in reaction did not have Mentana in their pedigree. The phenomena were exhibited in tests with both races.

Phytophthora canker of Russian olive. CARTER, J. C. *Phytophthora cactorum* (Leb. & Cohn) Schroet., obtained in 1945 from cankerous trunk tissue of a Russian olive on the University of Illinois campus, was used to inoculate greenhouse-grown olives in 1946, '47, '49, '50 and '51. Artificially infesting the soil in which 3 trees were potted gave no infections. From 51 stem inoculations, 40 cankers were produced. Stem cankers varied in length from 2–12 in. and 3 cankers girdled the stems and killed the trees. Cankers developed from April and May inoculations but not from June inoculations. First noticeable evidence of infection and canker production occurred 3–4 weeks after inoculation. Infected bark appeared as oval to elongate, depressed areas and the inner phloem was discolored brown to black. Cankers continued to enlarge until midsummer and the underlying wood was discolored brown to black. Brown to black streaks, produced in current season springwood, extended up and down the stems beyond the visibly diseased bark. Amber to brown gummy deposits of sap were conspicuous along the margins of some cankers. *P. cactorum* could be reisolated from cankerous tissues 4 weeks after inoculations were made, while the cankers were still enlarging. It could not be reisolated from cankers 6 to 21 months after inoculation.

Experiments with Actidione for control of cherry leaf spot (Coccomyces hiemalis). CATION, DONALD. In 1952 Actidione controlled leaf spot at 1 p.p.m. in 2 preharvest applications followed by 2 p.p.m. in 1 post harvest application. One application of 2 p.p.m. before harvest applied at any 1 of 3 dates from petal fall to 20 days later and another spray after harvest controlled perfectly. Unsprayed trees were 90 per cent defoliated by August 1. An early application of a 2 p.p.m. concentration gave some leaf injury but typical fruit dwarfing was observed only on 1 limb of 1 tree out of 20 under observation. Sprays of 2 p.p.m. and 10 p.p.m. resulted in total fruit abscission when applied in early bloom on the sprayed side of half-tree plots. Two applications of 2 p.p.m. prevented defoliation in a budwood orchard at East Lansing. Three to 4 applications in various combinations of 1 p.p.m. and 2 p.p.m. gave results superior to Bordeaux 4-6-100 in a commercial nursery. Applied after harvest as a 6× concentrate (12 p.p.m.) Actidione checked leaf spot. The remaining leaves held until final observation, Oct. 17.

Disease reaction in relation to inoculum dilution in bacterial blight of soybean. CHAMBERLAIN, D. W. The relationship between dilution of inoculum and disease reaction of soybean was studied in the greenhouse with *Pseudomonas glycinea* as the test organism. Inoculations were made with an atomizer, watersoaking the leaves with the bacterial suspension. The following concentrations of bacteria per ml. sterile water were used: 8,000,000; 800,000; 80,000; 8,000; and 800. There was a direct relationship between concentration of bacteria and the number of typical lesions induced on resistant and susceptible plants. Concentrations between 800,000 and 80,000 cells per ml. gave the most pronounced differential between resistant and susceptible varieties. Although the 8,000,000 concentration gave excellent differential reaction in the field, it occasionally induced too severe a reaction on greenhouse-grown varieties of proved field resistance. Reducing the bacterial load in the inoculum appears to compensate for this difficulty in blight-resistance testing work in the greenhouse.

Dissemination of Septoria linicola. CHRISTENSEN, J. J., L. HENDERSON AND M. ARAGAKI. Septoria linicola is readily disseminated into new localities by means of infested flax seed and by bits of diseased material present among the seed. In Minnesota, most seed lots of flax contain infected plant debris, often with numerous pycnidia, sometimes from 50 to 75 per sq. mm., and each pycnidium may contain from 1,000 to 20,000 spores. Although local spread of the pathogen occurs with splashing raindrops and with strong winds during rainy weather, the fungus is not adapted for dispersal by air currents because the spores ooze out in a gelatinous matrix. No spores of *S. linicola* were caught on glass slides exposed over severely infected flax fields. It was demonstrated repeatedly that at least 20 species of insects were important agents in the dissemination of the fungus. During moist weather legs and bodies of insects collected on diseased flax plants were literally covered with spores of *S. linicola.* Any creature that walks, runs, jumps, or crawls through infected fields when plants are wet is an agent in the spread of the fungus, because animals such as spiders, mice, frogs, birds, and dogs have been shown to disseminate spores. Flax plants inoculated by means of infested insects developed pasmo.

A method of identifying disease resistance suited to interspecific transfer. CLAYTON, E. E. Attempts to utilize disease resistance occurring in wild species may fail because the resistance selected is not suited to interspecific transfer.

Immunity inherited as a monogenic dominant is of critical importance with interspecific crosses. Ordinary tests with wild species of tobacco may not distinguish clearly between desirable and undesirable types of resistance and adequate F_2 generations are rarely obtained from interspecific crosses. The nature of the resistance must be evaluated by other means. For example, both *Nicotiana debneyi* and *N. longiflora* appear immune from black root rot. However, if their tetraploids with *N. tabacum* are tested, the debneyi-tabacum tetraploid reaction is still root rot immune, but the longiflora-tabacum tetraploid is only moderately resistant. It was concluded from this that the root rot resistance in debneyi was probably monogenic dominant immunity. This was later proved true. Tetraploids are not essential for this test. The F_1 glutinosa $\times$ tabacum grows normally, though sterile, and indicates that glutinosa mosaic immunity may be a monogenic dominant. The same result is obtained with glutinosa-tabacum tetraploid. The suggested procedure measures the expression of resistance in the presence of susceptibility. If the F_1 or tetraploid is less resistant that the resistant parent, the resistance in question is probably polygenic and in part recessive. Such resistance is slow and difficult to transfer interspecifically and usually cannot be transferred completely. Monogenic dominant resistance may be transferred both rapidly and completely.

Unsuitability of mechanical inoculation in determining the field reaction of potatoes to potato virus Y. CORBETT, M. K. AND L. C. PETERSON. Inbred lines of potatoes were mechanically inoculated in the greenhouse with 4 strains of virus Y. Those clones that gave no reaction and those that produced local lesions without systemic infection were planted in exposure plots in the field. Almost all became systemically infected. Virus obtained from some of these was used in the greenhouse for inoculation of healthy plants of one of the clones that appeared to be hypersensitive in the original tests. Local lesions resulted with no systemic infection. Clones that appeared to be hypersensitive or nonreactors to mechanical inoculations were inoculated in the greenhouse with the 4 strains of virus Y by means of aphids. Systemic infection developed in almost all of these plants, but not in comparable ones mechanically inoculated. Inoculation of the same clones by graft resulted in systemic infection. Application of indole acetic acid to petioles of inoculated leaves did not affect local lesion formation, but delayed leaf abscission. Systemic infection occurred in these plants, but not in comparable nontreated ones.

Etiology and control of a serious complex of diseases of conifer seedlings. COX, R. S. A disease complex—consisting of damping-off, root-rot, stem canker and needle blight—has long been a serious limiting factor in the production of conifer seedlings, particularly white pine and Douglas fir, at the State Forest Nursery, Lincoln, Delaware. Losses often amount to 75–100 per cent. The primary causal fungus is *Cylindrocladium scoparium*. A highly effective control program has been developed through a combination of soil fumigation and foliage sprays. Control of damping-off and root-rot was obtained with pre-seeding soil treatments of commercial grade formaldehyde (1-25 conc., 1 qt./sq. ft.) or chloropicrin (1 gal./1000 sq. ft.). Other soil treatments were less effective. Control of stem canker and needle blight was obtained with each of the following materials (plus Triton B-1956 spreader-sticker) applied monthly, May through September: Bordeaux mixture (8-8-100), Manzate (1½-100), Zineb (2-100), Ferbam (2-100), or Thiram (1½-100). Three mercury-containing fungicides (Puratized Agricultural Spray, PMAS and Calocure) caused serious needle injury, as also did the copper oxide-containing Robertson's Fungicide. Use of the combined treatments of soil fumigation and foliage spraying results in larger, sturdier plants in 2 years than was formerly possible in 3-4 years.

Comparative studies on species of ascochyta from okra, bean, and cotton in North Carolina. CROSSAN D. F. Cross-inoculations showed that fungus isolates from naturally infected host material that conformed to the descriptions of *Ascochyta abelmoschi* from okra (*Hibiscus esculentus*), *A. phaseolorum* from snap bean (*Phaseolus vulgaris*) and lima bean (*P. limensis*), and *A. gossypii* from cotton (*Gossypium hirsutum*) are all highly and equally pathogenic to snap bean, lima bean, cotton, and okra. All isolates produced pycnidia on all hosts. Comparative studies of isolates and herbarium material showed that the pathogens from the several hosts are morphologically very similar and are indistinguishable on the basis of spore size and shape. Variation in cultural characteristics appeared to be as great among isolates from one host as between those from any two hosts. These studies suggest strongly that the above mentioned species of Ascochyta described on these three hosts are identical. Furthermore, additional inoculations showed that isolates from each of these hosts are pathogenic to hollyhock (*Althaea rosea*), soybean (*Glycine max*), pepper (*Capsicum annuum*), and eggplant (*Solanum melogena*). This indicates that the morphologically similar species described on the latter hosts may also prove to be closely related to or identical with those described on okra, bean, and cotton.

Phytophthora blight of summer squash in North Carolina. CROSSAN, D. F., F. A. HAASIS, AND D. E. ELLIS. A destructive leaf and stem blight of summer squash (*Cucurbita pepo* var. *condensa*) has occurred sporadically in several plantings in the Coastal Plain and Piedmont areas of North Carolina since 1948. The disease is characterized by a soft, watery breakdown of leaf blades and petioles. Invasion of the stem results in rapid wilting and death of the plant. Fruits are also affected. The disease is severe during warm, humid weather and has caused complete loss of some plantings. A fungus tentatively identified as *Phytophthora capsici* has been isolated repeatedly from diseased squash plants and its causal relationship established by inoculations. Isolates of the fungus are highly pathogenic to pepper (*Capsicum annuum*) causing symptoms comparable to those produced by *P. capsici* on this host. Successful inoculations resulted when zoospore suspensions were placed in leaf axils and on leaf blades and petioles of 30-day old Early Prolific Straightneck squash plants. In other tests, rapid stem girdling and death resulted when the fungus was added to the soil in which healthy plants were growing.

Studies on the availability of oak wilt inoculum in Illinois. CURL, E. A. Results of field studies from October, 1952, through May, 1953, indicate that the greatest concentration of potential inoculum of the oak wilt disease in Illinois may be expected in March, April and May. Numbers of conidia and perithecia were estimated and viability of spores determined from samples taken from mycelial mats which were designated according to the extent of their deterioration as immature, mature, aging, old, and very old. Highest conidial counts were obtained in December, January, March, and April from immature and mature mats. Best germination was obtained with conidia collected from immature mats in March and April. Only 13 per cent of the mats examined contained perithecia. Viable ascospores were found on 3 per cent of these mats and only in October, November, April, and May. The largest number of perithecia per mat occurred in April and May on aging mats and ascospores from these perithecia germinated best. Mats which were sampled and left intact on standing trees in the early spring changed rapidly from immature to very old in 4-6 weeks, deterioration being hastened by the feeding of insects. During the winter mats usually required 10-12 weeks to reach the very old stage. Numbers and percentage of viable conidia decreased rapidly with increase in age of the mats.

Phytophthora trunk rot of Washington palms. DARLEY, ELLIS F., AND WESLEY D. WILBUR. A new disease of the native fan palm, *Washingtonia filifera*, has been found on numerous trees in yard plantings in Palm Springs, Cali-

fornia. Leaves of affected trees die rapidly and examination reveals that the trunks are partially or almost completely rotted. The rot is initiated at or near the ground and spreads rapidly. Trunks become soft and spongy and palms succumb in 2–3 months. Isolations from advancing margins of lesions have yielded consistently a species of *Phytophthora* tentatively identified as *P. parasitica*. The fungus has been isolated from soil about diseased palms but there is no evidence that roots are affected. Removing old leaves to enhance the appearance of palms causes small wounds on the trunk. Infection appears to take place through these wounds, the inoculum being splashed or washed onto the trunk in irrigation water. Artificial inoculations, accomplished through small wounds at the base of the trunk, have given positive results on 1-, 8-, and 14-year-old palms. One-year-old seedlings die in about 10 days, whereas older plants die in 2–3 months and develop typical trunk rot. No root infection has resulted from soil infestation studies.

The role of enzymes in the etiology of fusarium wilt of tomato. DAVIS, DAVID. A β-glucosidase capable of hydrolyzing salicin was detected in the vascular sap of tomato plants affected with Fusarium wilt disease but not in the sap of healthy plants. The vascular sap of diseased plants contained more oxidizable phenols than the sap of healthy plants. Mushroom phenol oxidase increased the discoloration of vascular sap of diseased but not healthy plants. The capacity of various phenols such as catechol, pyrogallol, and gallic acid to discolor the vascular elements of healthy tomato plants indicates the presence of a phenol oxidase in the host. *Fusarium oxysporum* f. *lycopersici* is apparently the source of the β-glucosidase found in the vascular sap of diseased plants. The pathogen grew as efficiently on equivalent concentrations of salicin as glucose, as the sole carbon source, in artificial culture. The evidence indicates that enzymatic hydrolysis of the host's conjugated phenols and subsequent oxidation and melanization of the free phenols are responsible for the vascular discoloration in the tomato Fusarium wilt disease. Wilt may be caused by fragments of high molecular weight thus formed or the free phenols may injure the mechanisms responsible for water movement.

The use of intergeneric grafts to demonstrate toxins in the Fusarium wilt disease of tomato. DAVIS, DAVID. Fusarium wilt disease symptom development in grafts of *Lycopersicon esculentum* stock on *L. esculentum* scions followed the pattern displayed in intact *L. esculentum* plants. Symptoms were apparent 14–21 days after inoculation of the roots with a washed spore suspension of *Fusarium oxysporum* f. *lycopersici*. The pathogen was isolated where symptoms were apparent whether below or above the graft union. Scions of *Solanum tuberosum*, *Solanum nibrum*, *Nicotiana tabacum*, and *Physalis alkekengi* grafted on *L. esculentum* (Bonny Best) stock showed vascular discoloration and severe wilt 5–9 days after inoculation of the tomato stock roots. The tomato stock was symptomless at this time, yet the pathogen was isolated from the tomato stem but not from the symptom bearing scions. The vascular elements of *Petunia hybrida* and *Capsicum grossum* scions did not become discolored 21 and 35 days respectively after inoculation of the tomato stock. This experiment demonstrates that a translocatable toxin(s) is produced by the host-parasite interaction in the Fusarium wilt disease. The differential responses of the scions of the various genera is attributed to difference in the reactants in the scions, or the presence of toxin inhibitors, or accelerators.

Amino-acids synthesized by lines of Ustilago zeae of different sex. DEVAY, J. E. AND E. C. STAKMAN. Chromatographic analyses of lines of *Ustilago zeae* belonging to several sex groups were made to determine possible association between sex and free or bound amino-acids synthesized in culture. There were quantitative and qualitative differences in the free amino-acid content of liquid shake cultures of different lines, but variability in their production was as great among lines of the same sex group as among lines from different groups. Cultures having the same amount of growth did not necessarily have the same culture pH or free amino-acid content. Acid hydrolysates of alcohol-extracted cells of these lines contained the following ninhydrin-reacting compounds: aspartic acid, glutamic acid ,serine, glycine, threonine, alanine, lysine arginine, proline, valine, tyrosine, leucine and/or isoleucine, cystine, methionine, histidine, and two unknowns. No marked differences were apparent in the bound amino-acids of lines of *U. zeae* from different sex groups.

Symptoms of bean yellow mosaic in red clover. DIACHUN, STEPHEN AND LAWRENCE HENSON. Bean yellow mosaic virus is one of the common viruses in red clover in Kentucky. It was recovered from 113 of 136 red clover plants (from 5 counties) showing several kinds of virus-like symptoms. A range of symptoms similar to those in naturally infected plants was induced in inoculated seedlings of Kenland red clover. Of 1051 plants inoculated, 563 developed mild or severe mottle or vein chlorosis; an additional 56 showed some veinal necrosis with or without chlorosis; of these 12 became systemically necrotic and died. Cuttings of selected clones of Kenland red clover also showed a similar range of symptoms when inoculated. Plants of 126 clones were inoculated. Symptoms varied from clone to clone, but within a clone the symptoms were alike. Plants of 95 clones developed vein clearing or mottle, from very mild to prominent, distorting, and stunting. In plants of 10 clones some form of necrosis accompanied mottle or vein necrosis. Plants in 13 clones developed only necrosis, often resulting in death. Plants of 8 clones showed no symptoms.

Ethylene, the cause of epinastic symptoms in fusarium wilt of tomatoes. DIMOND, A. E. AND PAUL E. WAGGONER. Ethylene or ethanol are possible causes of epinasty, an early symptom of Fusarium wilt of tomatoes. The amount of ethanol which *Fusarium* produces in culture and in the infected plant was estimated refractometrically from distillates of culture filtrate (0.65 per cent) and tracheal fluid (0.16 per cent). Uptake of such concentrations did not cause epinasty in tomato cuttings nor in healthy tomato plants confined with vapor from these solutions. Therefore ethanol is not considered responsible for epinasty. One thousand p.p.m. ethanol caused the "triple response" in Alaska pea seedlings, a response formerly considered specific for ethylene of biological origin. The fungus produces a triple response which varies with the carbohydrate substrate. However, cultures also produce a gas which reduces $KMnO_4$, reacts with bromine, and responds negatively to the fuchsin aldehyde reagent. Ethylene is the only known compound of biologic origin with these properties. Infected, but not healthy, tomato plants cause epinasty in indicator tomato plants when confined together. Tomato plants exposed to ethylene do not shed leaves. Therefore, ethylene production by *Fusarium oxysporum* f. *lycopersici* is demonstrated *in vitro*, and diseased plants produce sufficient ethylene to account for the epinasty of the Fusarium wilt syndrome but not for defoliation. Atomic Energy Commission support is acknowledged.

The imperfect stage of Microthyriella rubi, cause of flyspeck of apple. DURBIN, RICHARD D., LILY H. DAVIS, WILLIAM C. SNYDER, AND KENNETH F. BAKER. The previously unrecognized conidial stage of *Microthyriella rubi* has been isolated from the flyspecks on apples from California and Virginia. This dematiaceous fungus, *Zygophiala jamaicensis*, originally described on bananas and recently reported by us to cause greasy blotch of carnation, has a wide host range. Flyspecks develop on hosts in 15 families including apple, carnation, banana, blackberry, and 17 wild plants. When mature, these thyriothecia discharge ascospores that initiate infection. Ascospore isolates from apples and wild hosts produced the perfect and imperfect stages when inoculated on young apple fruit; Zygophiala developed on carnation. Conidial isolates from carnation produced flyspecks and the Zygophiala stage on apple, carnation, banana, honeysuckle, and *Rubus* sp. The Zygophiala, but so far not the mature sexual stage, developed in culture. The isolates were similar morphologically, but

varied culturally. No fungus of the *Leptothyrium pomi* type has ever been shown to be part of the cycle of *M. rubi*, nor has it been cultured or inoculated on a host. No pycnidial stage occurred in our material. The binomial, *L. pomi*, though now commonly used, was based on a misconception and should be set aside, pending demonstrated relationship.

Inheritance of resistance to Argentine races of Puccinia glumarum in the wheat "Chino 166". FAVRET, EWALD A. AND JOSE VALLEGA. Crosses between several susceptible varieties of wheat and Chino 166 and Chino 166 × Lin Calel, were tested in F2 in the field nursery at El Bolson, Rio Negro in 1950 against yellow rust. The determined races were classified as 30, 37 and a new one; none of them was able to attack Chino 166. Observations show that results are very near to those expected for a monohybrid segregation involving a dominant gene for immunity (resistant plants = 800; susceptible plants = 244; $x^2(3:1) = 1.47$; P = 0.2-0.3). The cross between Sinvalocho M. A. and Chino 166 was analyzed separately as the segregation was far from expected (resistant plants = 154; susceptible plants = 351). In this case, the resistance behaved like a recessive factor. This anomalous behavior could be explained in different ways but it might be due to the well known chromosomic instability of the susceptible parent.

The influence of the feeding habit upon the nematocidal action of Systox. FEDER, WILLIAM A. *Rhabditis sp.* is not killed by concentrations of Systox vapors lethal to Ditylenchus dipsaci when both are present simultaneously in treated bulb tissue. Free-swimming *Rhabditis sp.* is killed in 1 hour while free-swimming *D. dipsaci* lives 48 hours in 0.5 per cent aqueous suspension of Systox. *Rhabditis sp.* is killed in contact with bulb tissue, blotting paper, or *Rhabditis* infested tissue dipped in 0.5 per cent Systox. Partial kill results from exposure to vapors of the same concentrate. Forty-eight hours' exposure at 28°C. to vapors 0.05 per cent Systox is lethal to *D. dipsaci* in bulb tissue but no kill is observed after 186 hours at 20°C. Ten seconds in 0.05 per cent Systox is lethal to *D. dipsaci* whether the treated tissues are held at 20°C. or 28°C. The forward regions of *Rhabditis sp.* become blackened after 5 minutes in a dilute india ink suspension, while 24 hours elapses without similar blackening of the forward regions of free-swimming *D. dipsaci*. Apparently, both nematodes are killed by ingesting Systox. The vapors are not lethal, but supply plant cells with Systox upon which *D. dipsaci* feeds. *Rhabditis sp.* is also killed by feeding upon Systox-impregnated dead or inert material.

The structure and cytology of Ditylenchus dipsaci-induced "spikkels" in leaves of Narcissus pseudonarcissus. FEDER, WILLIAM A. AND JULIUS FELDMESSER. It has long been known that *Ditylenchus dipsaci* infections of *Narcissus* sp. result in "spikkeled" areas on leaves, sickle-shaped leaves, stunted unsaleable flowers and eventual destruction of the bulbs. The cytological abnormalities associated with these effects have been compared with data available for the better understood root-dwelling nematodes such as *Heterodera* spp. and *Meloidogyne* spp. The external leaf morphology and the raising of the "spikkeled" area above the leaf surface are the results of hypertrophy and hyperplasia in areas adjacent to nematode loci. Groups of affected epidermal cells enlarge and buckle away from underlying tissue forming loculate "spikkels" containing the nematodes. Giant cells are often found in areas containing *D. dipsaci*. These are similar in form and structure to those in roots caused by root-dwelling nematodes. These cells are characterized by heavily stained cytoplasm, smaller vacuoles and abnormally large, often pycnotic, nuclei. Mitotic activity was not observed. These data indicate that *Ditylenchus dipsaci's* mode of feeding is probably the same as that possessed by *Heterodera* and *Meloidogyne* and that "spikkels" are homologous with nematode galls.

A cytological study of the effects of the golden nematode, Heterodera rostochiensis, on tomato. FELDMESSER, JULIUS. The nematode larva enters growing secondary and tertiary roots during its second stage. Cells near the worm's path are compressed or destroyed and some cells adjacent to the worm's head begin developing abnormally, showing dense cytoplasm and thickened cell walls. Large syncytia (giant cells) become evident during the nematode's third stage. They develop in the cortex and in the stele causing discontinuity of some vascular elements and the retardation of differentiation of other vascular elements. Some external evidences of galling occur. During the fourth and fifth stages of the nematode's life cycle, giant cell areas become more extensive and individual giant cells become larger and more irregular in shape, cutting off greater areas of vascular tissues. The galled areas become larger, linearly and in girth. The processes involved in the formation of giant cells are fragmentation and dissolution of cell walls, coalescence of the cytoplasmic masses of the previously intact cells and the aggregation of the nuclei from these cells.

Factors in colonization of sclerotia by soil organisms. FERGUSON, JOHN. Nutrient-rich, viable sclerotia of plant pathogenic fungi are rarely colonized by soil organisms, whereas those killed by various soil treatments are rapidly decomposed. Organisms with potential value for biological control are easily selected by their ability to colonize living sclerotia. Viable and non-viable sclerotia of either *Sclerotinia sclerotiorum* or *Sclerotium delphinii* exhibited differential colonization when placed on field soil or on cultures in Petri dishes. Either intact or halved viable sclerotia generally remained free from other organisms, except occasional species of *Penicillium* and *Trichoderma*. Killing portions of a sclerotium permits the dead part to be overgrown. Apparently resistance is not dependent upon the rind, since cut surfaces of living sclerotia were not colonized. Because viability and resistance of whole or cut sclerotia of *S. delphinii* are unaffected by leaching for 8 hours in running water and/or by inhibiting their growth during exposure to 50°F., resistance does not depend on a water-soluble material or an actively respiring sclerotium. Those killed by various chemical and physical means were equally susceptible to colonization. Living and non-living sclerotial tissue was successfully differentiated by use of 2,3,5-triphenyltetrazolium chloride. Bacteria, fungi, protozoa, mites, nematodes, and small annelids were associated with killed sclerotia.

The origin and extent of a regional spore shower of wheat stem rust. FLETCHER, D. G., E. B. LAMBERT, K. M. NAGLER, AND E. C. STAKMAN. The appearance of primary infections of stem rust of wheat in eastern South Dakota, eastern North Dakota, northwestern Iowa, and western Minnesota between June 24 and 28, 1952, indicated that a comparatively uniform spore shower fell over the entire area earlier in June. Calculations based on estimates which by their nature cannot be precise indicate that approximately 600 lbs. of spores fell on this area of about 40,000 sq. mi. during the spore shower. The source of the urediospores appeared to be in central Oklahoma and south central Kansas where combining operations dispersed the spores into the air. Estimates based on the acreage of wheat and the severity of rust infection in the source area, assuming about 0.5 mg. of spores per culm, indicate that approximately 4000 tons of spores were produced within the area. An analysis of meteorological conditions shows that much of the air in the lowest 2–3, 3,000 ft. which passed over the source area during the second and third weeks in June moved northward into the spore-shower area where conditions were favorable for infection.

Comparison of fungicides for the control of strawberry leaf blight (Dendrophoma obscurans). FULTON, ROBERT H. AND DONALD CATION. Two control programs were compared on the Robinson variety which is very susceptible to infection by *Dendrophoma obscurans*. An eradicative program consisted of a single application in the spring when the new leaves were emerging from the crown of Tag 331 (phenyl mercury acetate), diluted to ½ pt. in 100 gal. Ferbam, Captan, and fixed copper were evaluated

as protectants, using 2 additional applications, pre-bloom and post-bloom. Unsprayed plots showed an average of 737 blighted leaf areas per 3 ft. of matted row. Tag 331 reduced this infection to 15.5, Ferbam 121.5, Captan 135.8 and fixed copper 192.5. Foliar injury was noted only in plots sprayed with Tag 331 where older infected leaves turned yellow and died. A slight black spotting appeared on the younger leaves. There was no apparent injury to the crown and the plants showed good recovery. Tag 331 and Elgetol (sodium salt of dinitro ortho cresol) were compared in a post harvest application about August 1. The number of healthy leaves resulting were as follows, checks (no spray) 15 per cent, Tag 331 (½ pt.) 73.8 per cent, Tag 331 (1 pt.) 82.2 per cent, Elgetol (½ gal.) 30 per cent and Elgetol (1 gal.) 28.8 per cent. Similar results were obtained with the same program the next season.

Resistance in tobacco to cucumber mosaic virus infection. FULTON, ROBERT W. Of 44 species of *Nicotiana* and over 350 accessions of *N. tabacum*, none showed tolerance or hypersensitivity to ordinary cucumber mosaic virus. The tendency not to become infected by small amounts of inoculum was then sought, using a necrotic strain of virus. This strain, apparently a chance mutant, was isolated from a stock culture. It produced only local necrotic lesions on most of 45 species tested in 14 families, but produced mosaic on *Vinca rosea*, which provided adequate amounts of inoculum. Properties and cross protection tests indicated its relationship to cucumber mosaic virus. Tobacco accessions were compared with Wisconsin Havana 38 in numbers of lesions produced per unit of leaf area inoculated. Most of 125 accessions developed approximately the same numbers of lesions as did Havana 38. Two consistently developed 10–12 times as many as Havana 38, and several developed only about 1/10 as many. The best of the latter, in replicated field tests near mosaic cucumbers, showed about 4 per cent infection at harvest as compared to 20.5 per cent infection of commercial varieties. Apparently resistance to infection by mechanical inoculation is effective also against naturally occurring, presumably aphid transmitted strains of cucumber mosaic virus.

A transfer of stem rust resistance from Triticum dicoccum variety Khapli to common wheat. FUTRELL, M. C. AND R. G. SHANDS. *Triticum dicoccum* Schulb. var. Khapli has been shown to be resistant to *Puccinia graminis tritici* race 15B. Khapli (n = 14) was crossed with a number of common wheats (n = 21). The F_1 plants of these hybrids were intermediate in response to race 15B of the stem rust pathogen. Severe chlorosis and necrosis of genetic origin resulted in the death of the F_1 plants of several Khapli hybrids at different stages of development. Reward × Khapli F_1 plants died in the seedling stage from this chlorophyll disorder. However, a selection of C. I. 12632 × Frontana crossed with Khapli produced F_1 plants that headed before the chlorophyll disorder occurred. Lines of this hybrid were stabilized for fertility and chlorophyll development by backcrossing the progenies twice to common wheat as the pollen parent. A number of the F_2 lines from the second backcross were resistant to race 15B of the stem rust pathogen when tested in the seedling stage under greenhouse conditions. Agronomic values of the lines have not been determined.

Races 49 and 139 of the wheat stem rust pathogen further complicate breeding programs. GIBLER, J. W., I. NARVAEZ M., AND T. ENCISO C. In most wheat breeding programs in North America where stem rust is a problem, the major emphasis during the past 3 years has been to develop varieties resistant to race 15B, and simultaneously retain resistance to the other common races. Races 49 and 139 began to increase in prevalence in Mexico during the summer of 1952, on the commercial varieties Kenya 324, Lerma, and to a lesser extent on Kentana, all of which are resistant to race 15B. Subsequent greenhouse and field tests indicate that all the Kenya wheats which have been shown to be resistant to race 15B, with the exception of Kenya 338AC2E2 and Kenya 338AA1A2, are susceptible to races 49 and 139. The latter Kenyas are resistant to races 15B, 49, and 139. Races 49 and 139 are currently (May 1953) second in prevalence to race 15B in central and northern Mexico. Since a large percentage of the acreage in these areas is planted to Lerma and Kentana, races 49 and 139 will probably soon become more prevalent than 15B. Races 49 and 139 are similar from a breeding standpoint. Many commercial United States, Canadian, and Brazilian varieties are resistant to both. A strong linkage exists in most Kenya varieties between the factors for resistance to race 15B and susceptibility to races 49 and 139.

Control of oak leaf-blister in Mississippi. GOODE, MONROE J. Oak leaf-blister, caused by *Taphrina coerulescens*, a common disease in most Southern states, is severe following cool, wet springs such as in 1953, though some disease is present every year. Infection of leaflets occurs just as the oak buds open, not before. Application of eradicant fungicides to dormant buds controls this disease. Applications made when buds are swelling and bursting are only partly effective; applications made after leaves have appeared are ineffective. Overwintering ascospores of *T. coerulescens* are killed by a variety of fungicides. A single spray application of 4-4-50 bordeaux, zineb (Dithane Z-78), nabam (Dithane D-14 + zinc sulphate), Vancide 51, Manzate, Phygon XL, Merculine, Orthocide 406, Cop-O-Zink, Microgel, or Tri-basic Copper Sulphate, at customary strengths using power equipment to reach and thoroughly cover dormant buds, gave good control in 1953 on trees that in 1952 showed 75–95 per cent of their leaves infected with blister. Adjacent unsprayed trees, and unsprayed portions of sprayed trees, serving as checks were severely diseased. To insure a minimum of disease, and where power spray equipment is available, oak trees should be given 1 spray, during the winter months when the buds are dormant. Sanitation, involving removal of all diseased, fallen leaves during the fall, in the absence of spraying, gives no useful control.

Pectic enzymes in the physiology of Fusarium wilt of tomato. GOTHOSKAR, S. S. AND R. P. SCHEFFER. Vascular browning and wilt in Fusarium infected plants was studied, using tomato cuttings and culture filtrates of *Fusarium oxysporum* f. *lycopersici*. The culture filtrates, obtained by growing the fungus on moist wheat bran and extracting with water, caused vascular browning and wilt in tomato cuttings. The active factor in the filtrate was heat labile and non-dialysable and its action suggested that an enzymatic reaction might be involved. Consequently various commercial enzyme samples were tested on tomato cuttings. Only those enzyme samples containing pectic enzyme activity were found to duplicate the action of the culture filtrates. A correlation between the severity of vascular browning and pectin methyl esterase activity was observed in the various commercial enzyme samples. An active pectic enzyme preparation was isolated from the culture filtrates by precipitation with alcohol. This enzyme preparation showed high methyl-esterase and low polygalacturonase activity and produced the typical wilting and vascular browning which are the most specific symptoms in diseased plants. The results indicate that pectin methyl esterase produced by the pathogen is an important factor in causing the characteristic wilt and vascular browning associated with Fusarium wilt of tomato.

Biotypes within race 7 of Puccinia graminis avenae. GOTO, S. Previous work in Canada and Minnesota indicated that the former rarity of race 7 of oat stem rust might be due to early telial development and consequent reduction of urediospore inoculum. In 1950, however, the prevalence of race 7, together with the related race 12, increased to 44 per cent of the isolates identified in the United States; and in 1951, to 76 per cent. Four isolates of race 7, obtained from the Federal Rust Survey, were compared on Jostrain, Victoria-Hajira-Banner, and Clinton × Ukraine C.I. 6537 oats under different light intensities and at 70–75°F. and 80–85°F. The 2 hybrids were susceptible to the 4 isolates at high temperature, but resistant at low temperature. Only 1 isolate, from Maine, formed telia within 11 days on these oats at high temperature. The Maine isolate also was differentiated from the other

isolates on Jostrain at 70–75°F.: it produced blotchy infection type 1 with 400 foot-candles of light and type X with 3000 foot-candles of light. At 80–85°F. Jostrain was susceptible to all 4 isolates; the Maine isolate formed telia on Jostrain more rapidly with high light intensity than with reduced light.

Incidence of the red clover vein-mosaic virus in Wisconsin clovers. GRAVES, CLINTON H., JR. The red clover vein-mosaic virus, incitant of Wisconsin pea stunt, has been repeatedly isolated from red clover, alsike clover, yellow sweet clover, white sweet clover, ladino clover, and black medic found growing both wild and cultivated within the major pea-growing sections of Wisconsin. With the exception of black medic this virus was found quite distinctive, in symptomatology, on these hosts. A study of virus incidence among the above named hosts was carried out in the pea growing areas during 1951 and 1952. In Dodge county during 1952, red clover fields exhibited an average incidence of red clover vein-mosaic from nearly 12 per cent in early season to about 22 per cent in late season (new seedings excluded), and of other viruses about 2 per cent with no significant seasonal changes. Red clover along the roadsides exhibited an average incidence of red clover vein-mosaic between 18 and 22 per cent, and of other viruses between 5 and 8 per cent with no significant seasonal changes. Incidence among alsike and sweet clover stands was somewhat less than that above, although still surprisingly high; among ladino and black medic, however, almost negligible. Other areas studied exhibited essentially the same trends, although incidence in more northern areas was considerably lower.

Host-parasite relationships in Septoria leaf blotch of barley. GREEN, G. J. AND J. G. DICKSON. The host-parasite relationships in Septoria leaf blotch of barley were investigated in leaves of resistant and susceptible varieties. Under optimum conditions host penetration was predominantly stomatal in both susceptible and resistant varieties. Penetrations were not observed frequently until 48 hours after inoculation. The mycelium was intercellular in the mesophyll tissue and pycnidia formed beneath the stomata usually on the upper surface of the leaves. In the susceptible varieties, Vantage and Montcalm, the mycelium developed extensively and caused the degeneration and death of the host protoplasts. Leaf cells of the resistant varieties, Atlas and Feebar, reacted quickly to the fungus. The first visible evidence of a reaction was a safranin staining fringe on the host cell walls contacted by the hyphae. As the reaction progressed a depression formed in the protoplasts around the points of contact and the cell walls thickened in the affected areas. These cells died slowly and apparently the hyphae also degenerated. The relative resistance of the varieties studied appeared to be dependent on the intensity of this interaction.

The inhibition of virus infectivity by synthetic polypeptides. GREEN, MAURICE AND MARK A. STAHMANN. In a manner similar to the inhibitor proteins from plants, synthetic lysine polypeptides reduce the number of local lesions produced by tobacco mosaic virus. The evidence demonstrated that polylysine combines with viruses, bacteria, erythrocytes, and proteins. The resulting complex arises from interactions between the polyvalent positively charged polylysine and appropriate negatively charged receptor groups. It seemed probable that polylysine would interfere with virus-cell combination *in vivo* by competing for acidic centers on the virus or host cells. The demonstration by Puck and associates that attachment of bacterial viruses to *E. coli* involves an ionic interaction supported this concept. Data shows that the multiplication of influenza, infectious bronchitis, Newcastle disease, and mumps viruses in embryonated eggs is blocked by low levels of polylysine. Interference with virus attachment could also occur when basic sites on viruses or cells are blocked. In support of this, polyglutamic acid was prepared which reduced local lesion production from tobacco mosaic virus. Results suggest that virus infection may be reduced by interfering with the attachment of virus particles to susceptible host cells through competition with suitable acidic or basic poly peptides.

Resistance of wheat to bunt associated with inability to produce chlamydospores. GRIFFITH, R. B., F. P. ZSCHEILE, AND J. W. OSWALD. Resistance of Baart wheat (susceptible to race T-1 of *Tilletia caries*) was compared to that of Baart 38 (resistant), grown in field, greenhouse, and laboratory. Both became heavily infected when chlamydospore-covered seed was germinated at 10° C. Unless plants were left in the cold under lights until the first foliage leaves had emerged, abnormal emergence from the coleoptile occurred. Baart plants grown to maturity under fluorescent lights were completely bunted. Baart 38 plants appeared completely resistant. Histologically, chlamydospores were found within apparently normal kernels of Baart 38 and mycelium was detected in all plants of both varieties at all stages of growth. In the greenhouse, Baart was 100 per cent and Baart 38 12 per cent bunted. In the field, Baart was 21 per cent bunted and Baart 38 not bunted. Growth to maturity under incandescent bulbs at 10-13° C. resulted in 94 per cent bunt in Baart and 50 per cent in Baart 38. These growth conditions prevented the expression of resistance in Baart 38. Inoculated Baart 38 plants were stunted at early stages in the laboratory. In greenhouse and cold room they were late-maturing. Resistance in Baart 38 is connected with inability of the fungus to produce chlamydospores rather than inability to infect and develop vegetatively within the plants.

Seed transmission, mode of overwintering and spread of bacterial canker of tomato caused by Corynebacterium Michiganense. GROGAN, R. G., AND JAMES B. KENDRICK, SR. Previous reports indicate approximately 100 per cent seed transmission of bacterial canker may occur. Studies in California have failed to show more than 1 per cent even with freshly harvested seed taken from fruits showing severe internal infection. Seed dipped in a virulent suspension of bacteria, as well as seed planted with freshly ground diseased plant material, likewise have shown 1 per cent or less diseased seedlings. Spraying young plants with a virulent suspension of bacteria and holding in a moist chamber 6–10 days have failed to produce infection. Successful infection has only been secured when the bacteria were introduced into the vascular elements by wounding the stems or roots. Controlled field trials, as well as many years' observation, indicate that field carry-over of the disease from one season to the next is unimportant. The disease will, however, survive from one season to the next in diseased plant material incorporated into plant bed soil. The plant bed-to-field transplant method often results in more than 50 per cent diseased plants, whereas direct field seeding has never shown more than a trace of canker. In California effective control of bacterial canker can be secured by direct field seeding.

New Era, a recently developed disease-resistant canning pea. HAGEDORN, D. J. A new disease resistant canning pea variety, named New Era, has recently been developed at the University of Wisconsin. This new pea is resistant to wilt (*Fusarium oxysporum* form *pisi* race *1* S. & H.), near wilt (*F. oxysporum* form *pisi* race *2* S. & H.), and bean virus 2, the causal agent of a widespread virus disease of pea in Wisconsin. New Era is an "Early Perfection" type canning pea, maturing 2 or 3 days earlier than regular Perfection varieties. It has been found to possess high yielding capacity and excellent canning quality. Tests widely separated geographically have indicated its ability to produce well over a range of environal conditions.

Effects of age of host and method of applying carborundum on virus infection in clovers. HAGEDORN, D. J., AND E. W. HANSON. Plants 4, 8, and 16 weeks old of red clover, alsike clover, crimson clover, and yellow sweetclover were inoculated by the rubbing method in greenhouse tests with red clover vein-mosaic virus, yellow bean mosaic virus, and a strain of alfalfa mosaic virus. In most cases, good infection was obtained in each age group. The percentage of plants showing symptoms for each age group varied with

the host and virus. Comparisons were also made of 3 methods of applying carborundum to the above clovers to facilitate inoculation with the 3 viruses. The methods used were: 1) sprinkling the carborundum on the surface of the leaves with a large salt shaker, 2) blowing the abrasive on the foliage with a powder blower, and 3) mixing the carborundum with the inoculum and rubbing both on leaves simultaneously. Each of these methods gave very good infection with all the viruses and hosts studied. However, the first method gave the highest percentage of infected plants with the yellow bean mosaic virus; the third method was most successful for the alfalfa mosaic virus. No single method was superior for all 4 clover species in the case of the red clover vein-mosaic virus.

Relative pathogenicity of seven species of Pythium on alfalfa and sweetclover seedlings. HALPIN, J. E., AND E. W. HANSON. Seven species of Pythium were tested in greenhouse experiments at 16°, 20°, 24°, and 28° C. to determine their relative pathogenicity on alfalfa and sweetclover seedlings. Plants were grown for 15 days in glass tumblers containing a mixture of autoclaved white silica sand, sterile distilled water, a little agar, and the fungus to be tested. All tests included checks. Experiments were repeated 3 times with alfalfa and twice with sweetclover using 4 replications at each temperature for each trial. *Pythium debaryanum* Hesse, *P. ultimum* Trow., *P. irregulare* Buisman, and *P. splendens* Braun were very pathogenic on both hosts, killing all seedlings prior to emergence. *P. paroecandrum* Drechsler was less pathogenic but killed most seedlings prior to emergence and caused severe root rotting, stunting, spotting of the hypocotyls and cotyledons, and damping-off of emerged plants. *P. arrhenomanes* Drechsler had no significant effect on stand and caused only slight root discoloration. *P. rostratum* Butler was non-pathogenic. The pathogenic species incited disease over the entire temperature range of 16°–28° C. Alfalfa and sweetclover were about equally susceptible and reacted in a similar manner to the isolates tested. Both were more susceptible than red clover to all isolates except *P. rostratum* and *P. arrhenomanes.*—Cooperative investigations between the Wisconsin Agricultural Experiment Station and the Division of Forage Crops and Diseases, U. S. Department of Agriculture.

Nematode resistance in pepper. HARE, W. W. Previous tests with pepper varieties and strains in soil infested with root-knot nematodes indicated high but incomplete resistance in certain peppers. Pure cultures of *Meloidogyne incognita, M. incognita* var. *ascrita, M. arenaria, M. javanica,* and *M. hapla* were introduced into sterilized soil and increased by growing Southland tomatoes. Oakview Wonder and M81A, representing the susceptible group of varieties in previous experiments; Red Chili and M41, representing the intermediate resistant group; and Santanka ×S and M152B, representing the resistant group, were grown following the tomatoes. After 77 days plants were classified for nematode injury and indices calculated for each variety for each nematode population. Indices for each variety in sterilized soil were zero. *M. javanica* caused no injury to any variety. *M. arenaria* caused slight injury to Oakview Wonder, M81A, and M41. *M. incognita* and *M. incognita* var. *acrita* were severe on Oakview Wonder and M81A, moderate on Red Chili and M41, and did not injure M152B. *M. incognita* var. *acrita* produced a trace of injury to Santanka ×S but this variety was not injured by *M. incognita. M. hapla* was moderate on Red Chili and severe on all others.

Relative Susceptibility of Barley Varieties to Pythium graminicola Subr. HASKETT, W. C., AND H. C. MURPHY. Thirty-four varieties and hybrids of spring barley were tested under greenhouse conditions for resistance to root necrosis caused by *Pythium graminicola* Subr. Tests were made by growing the varieties in autoclaved soil and in soil infested with pure cultures of the pathogen after being autoclaved. Plants were removed from the soil 15 days after planting and washed in tap water. The average top length, root length, and wet weight of roots from each variety, both inoculated and noninoculated, were obtained. The final disease index was determined by dividing the measurements of the healthy plants by the respective measurements of the diseased plants. The wet root weight values were the most satisfactory indication of host response to *P. graminicola.* Varieties exhibiting greatest resistance were Montcalm, CI 7149; Pillsbury, CI 7166; Moore, CI 7251; and Trebi, CI 936. Among the most susceptible varieties were Odessa, CI 182; Artic, CI 1470; Vantage, CI 7324; and Minn. 37-54, CI 7256.

The pathogenicity of races 11, 49, 125 and 139 of Puccinia graminis tritici to Kenya wheat varieties and their derivatives. HAYDEN, E. B., D. H. SMITH, AND E. C. STAKMAN. Certain Kenya wheat varieties, principally Kenya 58 and Kenya 117-A, have been used as sources of resistance to race 15B of *Puccinia graminis tritici,* although their stem rust resistance is not entirely adequate under some environments. Races 11, 49, 125, and 139 have attacked both seedlings and adults of Kenya 58, Kenya 117-A, and the Kenya derivatives Kentana 52 and Lerma over a wide range of temperatures. Although these races are not prevalent they are widely distributed in the United States and Mexico. Should large acreages of these Kenya wheat derivatives be grown, these or other races may increase and become even more destructive than race 15B. Kenya 338 A. A. 1. A. 2. and Kenya 338 A. C. 2. E. 2. have been highly resistant at 68–72° F. and at 80–85° F. to all the isolates of races 11, 49, 125, and 139 to which they have been tested. Varieties deriving their stem rust resistance from Vernal, Frontana, Thatcher, and Timstein have been highly resistant to these 4 races although they are at least moderately susceptible to race 15B. Obviously intensive testing of parental material and promising hybrids to all available races and biotypes of *Puccinia graminis tritici* is desirable.

Fungi associated with diseases of peanuts on the market. HEIBERG, BARBARA C., AND G. B. RAMSEY. About 2000 carloads of shelled peanuts are unloaded in Chicago each year. Most of these peanuts are Spanish and Runner but occasional lots are Virginia. At intervals during the past 10 years samples of damaged peanuts from approximately 100 cars were studied to determine the cause of discoloration and decay of peanuts on the market. Most decay was found in the Runner type. Typical specimens of peanuts showing various kinds of damage were photographed and cultured. In this study 685 cultures were made and of this number 601 yielded fungi and 84 were sterile or yielded saprophytic bacteria. Of the fungus cultures 20.2 per cent were *Diplodia natalensis,* 15.8 *Penicillium* spp., 13.1 *Rhizopus* spp., 8.8 *Aspergillus* spp., 6.0 *Macrophomina phaseoli,* 4.1 *Alternaria* spp., and 4.0 per cent *Trichothecium roseum.* Other fungi including *Rhizoctonia solani, Sclerotium rolfsii, Papulospora* sp., *Trichoderma* spp. and *Syncephalastrum* sp. were isolated less frequently. Many of these organisms have been reported to cause discoloration and decay of peanuts in the field and during curing. The decay and discoloration found on the market represent concealed damage that had been overlooked during processing and grading. Pathogenic and saprophytic organisms also developed under moist conditions during storage and transit.

Effects of ethylene dibromide on nematodes associated with a root rot of southern pine seedlings. HENRY, BERCH W. Nematodes were obtained from roots and rhizosphere of longleaf pine seedlings from ethylene dibromide treated and untreated nursery beds by the Baerman funnel technique. Populations consisted of *Acrobeloides* spp., *Tylenchus* sp., *Ditylenchus* sp., *Aphelenchoides* sp., *Panagrolaimus* sp., *Diplogaster* sp., *Pratylenchus* sp., *Zeldia* sp., *Discolaimus* sp., and *Dorylaimus sp.* No nematodes were found in treated beds prior to seeding (3 weeks after treatment) and very few through the subsequent 4 weeks. At 5 weeks after seeding the population equalled, and by 8 weeks it was tenfold (2500 nematodes/100 cc. rhizosphere soil), that in the untreated beds. By 20 weeks after seeding it had declined to 1.5 times that of the untreated beds. The increase in total populations in treated beds consisted entirely of *Acrobeloides* spp., which are not considered pathogenic.

Root rot was severe in the untreated and very light in the treated beds. It is proposed that the early protection from nematodes afforded by ethylene dibromide enables the pine seedlings to establish root systems before the onslaught of the root rot and that the rapid increase of nonpathogenic species in treated beds has no practical bearing on root rot control.

Host relations and spread of the pine pitch canker disease. HEPTING, GEORGE H., AND ELMER R. ROTH. Inoculation experiments have established that the fungus *Fusarium lateritium* f. *pini*, which causes the pine pitch canker disease, will attack, in addition to Virginia, shortleaf, slash, and longleaf pine already reported, Scotch, pitch, and Table Mountain pine. Inoculations on the following species failed to produce lesions: loblolly, pond, white, red, and Japanese red pine; white, Sitka, and Norway spruce; and eastern hemlock. The disease is now known in 5 counties in Virginia, 12 in North Carolina, 2 in Tennessee, 8 in South Carolina, 15 in Georgia, 38 in Florida, 2 in Alabama, and 1 in Mississippi, and was found to be abundant in Haiti on *Pinus occidentalis* in February 1953. The percentage of Virginia pines cankered on 2 study plots in North Carolina rose from 9 and 6, in 1948, to 14 and 19, respectively, in 1951, with almost no change in 1952. There is some evidence that lesions resulting from insect feeding can become infection courts of the pitch canker fungus, but not the sole avenue of infection.

Yellow mosaic of grapevines and evidence of retention of the virus in the soil. HEWITT, WM. B., AND CHARLES J. DELP. Formally reported as grape mosaic, this disease is characterized by yellowing of the leaves of the young shoot tips in 120 varieties and 4 species of grapevines. Other symptoms include various types of leaf mottling and blossom shelling. The mottling varies from flecking about the small veins to pronounced clearing and banding of the larger veins and from small variegations to complete speckling of the leaves. Chip buds from separate canes showing only 1 symptom type grafted into healthy vines transmitted the entire complex of symptoms. Diseased vines in most vineyards occur in compact groups. Only 3 seasons mapping of 2 such groups showed spread to bordering vines. Spring plantings of young disease free vines in soil where old mosaic diseased vines had been removed the previous fall developed yellow mosaic the second season after planting. Vines planted the same time outside but adjacent to infested soil did not develop the disease. The mode of spread in the soil is not known.

Effect of various chemicals on infection by Rhizoctonia solani and Verticillium albo-atrum. HILBORN, M. T. Rhizoctonia and Verticillium infection of potato was not prevented by 10 days' watering with solutions of indole acetic acid, naphthalene acetic acid, 8-quinolinol sulfate, 1182 and 1207. Rhizoctonia infection of potato stems was reduced considerably by Actidione, Bacitracin, Polymixin, Rimocidin, and Streptomycin, and somewhat by 8-quinolino sulfate, Magnamycin, Penicillin, and Thiolutin, and was slightly increased by Aureomycin, Chloromycetin, and Terramycin, following 72 hours' intake of these materials. Verticillium infection of potato stems was reduced considerably by all materials except Aureomycin, Magnamycin, and Terramycin. Rhizoctonia infection of lettuce in sand was reduced completely by Magnamycin, considerably by Rimocidin and Thiolutin, somewhat by Streptomycin and Chloromycetin, and none by Aureomycin, Polymixin, and Terramycin, following 5–10 days watering with the materials. In parallel series, Rhizoctonia infection of tomato plants was reduced considerably by Polymixin, Rimocidin, Thiolutin, and Streptomycin, somewhat by Bacitracin and Penicillin, and none by A6786-101, Aureomycin, Chloromycetin, Magnamycin, and Terramycin, while Verticillium infection of tomato plants was reduced considerably by all materials except Chloromycetin and Magnamycin. Different effects from the same material on the same pathogen in different hosts, indicates a complex reaction in the plant.

Virus activity and host tissue growth on synthetic media with different levels of nitrate, phosphate, and potassium. HILDEBRANDT, ALBERT C., A. J. RIKER, AND JEAN L. WATERTOR. *Nicotiana tabacum* tissue of crown-gall origin containing tobacco mosaic virus that grew *in vitro* 3 years on synthetic media was the basic tissue. Growth was measured as wet weight, and virus activity as numbers of local lesions. Over 7500 cultures were employed. With $NaNO_3$ (for nitrogen) at 0, 0.1, 0.4, 1.6, 6.4 or 25.6 gm. per liter, growth developed poorly without $NaNO_3$, optimally with 0.4 or 1.6, and was inhibited by 25.6 gm. per liter. Virus activity increased with concentrations to 1.6 or 6.4 gm. per liter. KCl (for potassium) supported best growth at 0.4, good growth with 6.4 or no KCl, and inhibited growth at 25.6 gm. per liter. The virus activity was relatively constant except at 25.6 gm. per liter the number of lesions was about half that at lower concentrations. Cultures with 0, 0.0033, 0.033, 0.33, 3.3 or 33 gm. per liter NaH_2PO_4 (for phosphate) grew optimally with 0.033, or 0.33 gm. per liter, moderately without NaH_2PO_4, and were inhibited at 33 gm. per liter. Virus activity gradually diminished with increased NaH_2PO_4.

Chemicals for therapy of oak wilt. HOFFMAN, P. F. Chemicals were applied to seedling and sapling red, live and black oaks as soil and foliage applications immediately following symptom expression of oak wilt in 1952 and 1953 in the greenhouse and field. Urea, lime, oxyquinoline sulfate, Monsanto 4370, Vancide 51, Monsanto 4367, oxyquinoline benzoate and undecylenic acid gave the greatest reduction in disease of 87 chemicals tested. Effectiveness of treatment was rated by the number of surviving trees, percentage of crown exhibiting symptoms, and time required for a tree to succumb. Chemotherapeutic effect was not limited to any 1 group of chemicals and was independent of fungitoxicity. The Monsanto chemicals are known translocatable substances having a low order of fungitoxicity. Vancide 51, undecylenic acid, and the oxyquinoline derivatives are potent fungicides. Lime and urea are nutrients. Both foliage and soil applications gave significant results. Liming raised the internal pH of oak about 1 unit. *Endoconidiophora fagacearum* was reisolated from all trees sampled, both diseased and recovered. Vascular streaking was present in trees showing normal foliage following recovery from oak wilt. Dieback of tops was variable in recovered trees. Recovery of 75 per cent of infected trees has been obtained in some experiments.

The sting nematode breaks resistance to cotton wilt. HOLDEMAN, Q. L., AND T. W. GRAHAM. Soil cultures of the sting nematode were established in autoclaved soil. A mixture of oat and soybean was used as host plants to increase nematode populations. A duplicate lot of soil received the same treatment throughout but was kept free of sting nematodes. The 2 soil lots were sub-divided and Fusarium inoculum was added to give the following treatments: 1) nematode soil, 2) nematode soil plus Fusarium inoculum, 3) control soil plus Fusarium inoculum, 4) control soil alone. Each treatment contained soil lots planted to susceptible cotton and to resistant cotton. Three separate experiments were set up using Fusarium isolates from different sources. The resistant cotton succumbed to wilt only when the sting nematode and Fusarium were present in combination. The susceptible variety showed a greater percentage of wilt than the resistant variety. With Fusarium alone there was no wilt on the resistant cotton and a very low percentage of wilt on the susceptible cotton.

The tendency of some types of tobacco to escape infection by tobacco-mosaic virus. HOLMES, FRANCIS O. The French investigators Schwartz and Cuzin reported in 1951 that a line of tobacco known as Java Isère × Cabot was of low susceptibility to infection by tobacco-mosaic virus. In the present investigation this finding has been confirmed. Moreover, 2 genes formerly known in connection with other types of resistance to the same disease have been shown to determine similar tendencies to escape infection. The dominant gene *N*, controlling hypersensitivity, and a recessive gene necessary for tolerance, presumed to be r_{m1} of Nolla, both confer this kind of resistance. They have been shown to segregate independently of each other and to act additively

when combined in a single plant. Plants carrying the 2 genes show few and small necrotic primary lesions and no secondary lesions after inoculation with the distorting strain of tobacco-mosaic virus.

Correlation of resistance to eight Pythium species in seedling corn. HOOKER, ARTHUR L. Several *Pythium* species may incite a seed decay and seedling root rot of corn. The relative resistance of 45 monoploid derived diploid lines and 3 synthetic varieties of corn to pure culture inoculations of 8 *Pythium* species was measured in quartz sand over sterilized soil at 10° C. Correlation coefficients measuring the similarity of reaction of these corn strains to different species were as follows: *aphanidermatum* and *arrhenomanes*, .659**; *aphanidermatum* and *debaryanum*, .659**; *aphanidermatum* and *graminicolum*, .634**; *aphanidermatum* and *irregulare*, .909**; *aphanidermatum* and *paroecandrum*, .906**; *aphanidermatum* and *rostratum*, .381**; *aphanidermatum* and *splendens*, .893**; *arrhenomanes* and *debaryanum*, .593**; *arrhenomanes* and *graminicolum*, .680**; *arrhenomanes* and *irregulare*, .725**; *arrhenomanes* and *paroecandrum*, .744**; *arrhenomanes* and *rostratum*, .736**; *arrhenomanes* and *splendens*, .667**; *debaryanum* and *graminicolum*, .686**; *debaryanum* and *irregulare*, .739**; *debaryanum* and *paroecandrum*, .831**; *debaryanum* and *rostratum*, .385**; *debaryanum* and *splendens*, .799**; *graminicolum* and *irregulare*, .751**; *graminicolum*, and *rostratum*, .436**; *graminicolum* and *splendens*, .675**; *irregulare* and *paroecandrum*, .958**; *irregulare* and *rostratum*, .431**; *irregulare* and *splendens*, .889**; *paroecandrum* and *rostratum*, .474**; *paroecandrum* and *splendens*, .910**; and *rostratum* and *splendens*, .410**. Differences among corn strains and correlation coefficients were all statistically highly significant.

Differential action of compounds on spore germination and hyphal growth. HORSFALL, JAMES G., AND SAUL RICH. Fungicides are aimed at inhibiting a fungus, either its growth or its spore germination. Both functions are inhibited by such compounds as phenols, quinones, quaternary ammoniums, thiocarbamates, and heavy metals. Numerous compounds in our tests have inhibited hyphal growth of *Sclerotinia fructicola* on agar without affecting spore germination in water, however. Among these are hydrocarbons such as acenaphthene, diphenyl, naphthalene, (also chlorinated naphthalene), fluorene, phenanthrene, and α-hexachlorocyclohexane. Another group comprises heterocyclic compounds; several quinolines, methylated pyridines, piperidine, phenothiazine, phenoxthin, dibenzothiophene, and indole. Aniline derivatives, thiourea, and phenylurethane act in the same way. Perhaps the most striking difference between mycelial growth and spore germination is that the former necessitates nuclear division; the latter probably does not. Many of the compounds listed are known to affect nuclear division. It is interesting, however, that colchicine is inactive. The only compound so far discovered that inhibits germination in water without inhibiting growth on agar is aconitic acid. Little is known of its action except 1) that its close relative, citric acid, affects neither process and 2) that pH is not involved.

Bluegrass "going-out" control with phenyl mercurials. HOWARD, FRANK L. Injury from *Helminthosporium vagans* is serious each year on bluegrasses in New England, particularly from March through May. This blight is critical in the maintenance of Kentucky bluegrass (*Poa pratensis*) turf, since more than 90 per cent may "go out" in a single season, permitting invasion by crabgrass and chickweed. Foliage of the Merion variety is only slightly injured by the leaf spot phase, whereas the fatal crown necrosis or foot rot occurs on Kentucky, Delta, rough-stalked (*P. trivialis*) and Canada (*P. compressa*) bluegrasses in order of decreasing susceptibility. A comparison of fungicides on plots of Kentucky and Merion bluegrass has shown that sprays containing phenyl-mercury-compounds when applied at the rate of 1.6 ozs. of 10 per cent active at 5 gals. water/1000 sq. ft. gave outstanding control. When day temperatures exceed 80° F., foliar injury can be minimized by evening treatment. Plots with more than 50 per cent diseased plants on April 1 had less than 5 per cent disease on April 29, following applications on April 1 and April 14. Concurrently, disease increased in untreated adjacent plots above 90 per cent. The eradicant-protectant action of the mercurials appears to be more effective than cadmium-chromium complexes, cycloheximide, or iron sulfate. Early season application is essential.

Ascospore longevity of the oak wilt fungus as affected by temperature and humidity. JEWELL, FREDERICK F. Ascospores from cultures of *Endoconidiophora fagacearum*, were collected on sterile glass beads, placed in sterile Petri dishes, and stored in desiccators over saturated solutions of certain salts to maintain the desired constant relative humidities. Sixteen combinations of temperatures and humidities were used: 10°, 20°, 25°, and 30° C., and 93 per cent, 81 per cent, 51 per cent, and 20 per cent relative humidity. Tests for viability were made first at 7 days and at monthly intervals thereafter. Results indicate that low temperature and low relative humidity favor longevity. At 10° C. and at 20 per cent and 51 per cent relative humidity the ascospores were viable after 6 months, while at the higher humidities the spores lived from 90 to 150 days. At 20° C. the spores at 20 per cent relative humidity were viable at 6 months, while spores at the 3 higher humidities did not germinate at 60 days. At 25° C. spores at 20 per cent relative humidity lived approximately 120 days, while spores at the 3 higher humidities lived less than 30 days. At 30° C. spores at 20 per cent relative humidity lived approximately 60 days, while at 81 per cent and 51 per cent relative humidity the spores lived less than 30 days, and at 93 per cent relative humidity the spores lived 3 days.

Autumn treatment of soybean seed. JOHNSON, H. W. AND R. A. KILPATRICK. Experiments conducted at Stoneville, Mississippi, in 1947-48 and 1951-52 showed that application of seed treatment chemicals to soybean seed in the autumn results in statistically significant increases in seedling emergence over that obtained from non-treated seed, when both are planted in the field the following spring. In the 1947-48 test involving 4 varieties, 9 treatments and 2 storage conditions, stand improvement ranged from 13 to 18 per cent for the treatments Methocel plus Spergon, Spergon, New Improved Ceresan, Arasan, and Methocel plus Arasan for seed stored under constant temperature and humidity, and from 10 to 12 per cent for the treatments Spergon, Arasan, and Methocel plus Arasan for seed in common storage. In the 1951-52 test involving Dorman soybeans, 7 treatments, and common storage, stand improvement ranged from 13 to 24 per cent for the treatments Spergon, Spergon SL, Arasan, Vancide 51ZW, Arasan SF, and Vancide 51. These results have practical significance, since farmers in the Delta area are usually too busy in the spring to treat soybean seed, and processors prefer to treat in the autumn, when the soybean seed is cleaned, rather than handle the seed a second time in order to treat it in the spring.

Influence of nutrition, potato varieties and isolates of Alternaria solani upon disease development. JONES, E. D., AND H. M. DARLING. Pathogenicity of 29 *Alternaria solani* isolates collected in Wisconsin ranged from mild to severe on Triumph potato and Bonny Best tomato. Isolates highly pathogenic on potato were not necessarily virulent on tomato. All isolates sporulated on potato plants; few sporulated *in vitro*. Controlled nutritional studies showed low levels of nitrogen and magnesium accentuated symptoms on Triumph. Disease severity on plants in low levels of potassium and phosphorus was less than in low levels of nitrogen and magnesium, but considerably greater than in low calcium or balanced solutions. Differences between the disease indices of deficiency treatments and balanced solutions were highly significant except for low calcium. Triumph plants in the field in the post-blossom stage were most susceptible, followed by the blossom and pre-blossom stages respectively. Nitrogen and magnesium decreased with age in potato leaf tissue collected from commercial fields. Potassium was also highest at pre-blossom stage, but lowest at blossom stage. Lower leaves of potato were

lowest in nitrogen at the 3 blossom stages. Disease-free potato plants showed higher nitrogen, potassium, and magnesium content than diseased plants. Twenty-seven commercial potato varieties showed a wide range in susceptibility both in greenhouse and field.

Efficacy of certain organic compounds in control of bean powdery mildew under laboratory conditions. KEIL, H. L., H. P. FROEHLICH, AND F. B. MAUGHAN. Polysulfide compounds studied under laboratory and greenhouse conditions in protectant type powdery mildew (*Erysiphe polygoni*) tests as sprays show the following descending order of effectiveness: tetramethylthiuram trisulfide (Lo-737), tetramethylthiuram tetrasulfide (Lo-765), ethylenebisthiuram tetrasulfide (He-257), tetramethylthiuram disulfide (O-51), ethylenebisthiuram trisulfide (Lo-738), and ethylenebisthiuram disulfide (He-183). Dinitrocaprylphenyl crotonate (Karathane) appears to be at least comparable to Lo-737 in these tests. Small quantities of wettable surphur give practical control of mildew but serious foliage injury results. In dust tests Karathane was found to be much superior to the polysulfide compounds. It gives perfect control on both leaf surfaces with 1½ per cent, 3 per cent, and 5 per cent dust while the polysulfide compounds showed some control on the upper surface only. However, subsequent testing showed that just as effective control could be obtained with the polysulfides if heavier deposits of dust were used. Preliminary studies indicate that Karathane and He-257 are quite effective in eradicating active powdery mildew lesions at 1 lb. and ½ lb. Lo-737 and Lo-765 appear to have some eradicant properties at the 1 lb. level, while at the same concentration Lo-738 and O-51 appear ineffective.

The influence of environment on the physiology and pathogenicity of Pythium species on corn seedlings. KENDRICK, E. L. The relative pathogenicity of *Pythium* species, concerned with seed decay and seedling blight of corn, was compared at 4°, 8°, 12°, and 16° C. *P. aphanidermatum* incited severe blighting at all temperatures; *P. irregulare*, *P. debaryanum*, and *P. ultimum* were similar and somewhat less pathogenic; *P. arrhenomanes* was more pathogenic at 12° and 16° than at the lower temperature; *P. splendens* was moderately pathogenic throughout. *P. arrhenomanes* and *P. debaryanum* were considerably less pathogenic than *P. aphanidermatum* and *P. irregulare* when incubated at 8°. Under high moisture at 8°, *P. debaryanum*, *P. irregulare*, and *P. aphanidermatum* were similar in pathogenicity; as moisture was decreased, *P. debaryanum* dropped off markedly in pathogenicity while *P. irregulare* and *P. aphanidermatum* remained pathogenic. Resistant corn inbreds accentuated the differences in pathogenicity between species. Xylan, pectin, glucose, and sucrose were used as carbon sources with asparagine and sodium nitrate as nitrogen sources in synthetic media to compare mycelial growth and compounds produced at the 4 temperatures.

TMV concentration in Marglobe tomatoes as affected by nitrogen availability. KENDRICK, J. B., JR., A. W. TROXEL, AND R. B. HARDING. Marglobe tomatoes were seeded in nutrient sand cultures, grown at deficient, normal, and excessive levels of nitrogen for about 28 days before and for 30 days after inoculation with TMV. TMV was extracted by the standard ultracentrifugation technique from a sample of terminal leaflets from each infected plant. Virus concentration was determined by quantitative absorption of ultraviolet light at 265 mμ. Plants at deficient (3 and 7 p.p.m.) and excess (500 and 1000 p.p.m.) levels of nitrogen contained, respectively, 2.7, 4.8, 6.4, and 8.2 per cent total nitrogen based on dry leaf weight. Leaves of plants receiving adequate nitrogen (70 p.p.m.) contained 5.4 per cent nitrogen. TMV-infected plants under deficient and excess nitrogen yielded stunted plants with virus concentrations significantly different from those receiving adequate nitrogen. TMV concentration measured as mg. of virus protein per gram of fresh leaf tissue averaged 34 per cent less in N-deficient plants and 50 per cent more in N-excess plants than that in plants with adequate nitrogen. Between 370–400 mg. virus protein was produced for each gram of total leaf nitrogen. This seems to indicate a direct relationship between nitrogen availability and virus elaboration rather than a direct relationship between plant growth and virus production.

Stemphylium leaf spot of alfalfa in Minnesota. KERNKAMP, M. F., AND R. R. NELSON. Etiology and epidemiology of leaf spot of alfalfa caused by *Stemphylium botryosum* were studied in the field and greenhouse, and the perfect stage of the organism was identified as *Pseudoplea briosiana*. Relative humidity of 100 per cent for at least 12 hours is essential for initial infection, and temperatures from 16° to 26° C. are most conducive to further development of the disease. When these conditions occur the disease can be epidemic in Minnesota. *S. botryosum* infects leaves, stems, and petioles, causing defoliation, wilting, and dwarfing of alfalfa. Entire stems are killed and seeds can become infected, but the greatest loss is from defoliation. In the greenhouse approximately 50 per cent of the leaves will fall in less than 4 weeks. Defoliation depends on position and number of lesions, petiole infection, size of lesions when correlated with position, and age of plants. Virtually all the leaflets drop if lesions are on the petioles or basal area of the leaflets. Alfalfa is most susceptible when the plants are in the flowering stage. The fungus penetrates directly or enters through stomata and grows intercellularly. There are pathogenic races of the pathogen.

Purple stain of legume seeds caused by Cercospora isolates. KILPATRICK, R. A., AND H. W. JOHNSON. Purple stain of soybean seeds is caused by *Cercospora kikuchii*. To determine if other *Cercospora* isolates could cause a similar discoloration, surface sterilized soybean seeds, that remained fungus free for 48 hours were inoculated by placing agar fragments containing the desired isolate on each seed. Of 34 isolates of Cercospora from 18 different hosts, 31 produced a purple discoloration of soybean seeds that was indistinguishable from the discoloration caused by *C. kikuchii*. Fifteen of the isolates also were tested on white seeds of snapbean, lima bean, lupine, and cowpea plated on an agar medium. Only one failed to produce a purple discoloration. During the course of these studies, 22 *Cercospora* isolates from 14 different hosts sporulated when mycelial fragments were streaked in steamed carrot leaf-decoction agar in Petri dishes just before the medium hardened. Sporulation was observed 3–5 days after streaking and occurred at the edges of small colonies. Stock cultures, including *C. kikuchii* and *C. capsici*, sporulated on this medium. When the agar medium was prepared from a decoction of pressure sterilized carrot leaves, sporulation did not occur.

Corn seedling blight in dry soil. KOEHLER, BENJAMIN. In greenhouse tests at 70° F., retarding corn germination through water deficiency increased seedling blight. Dark silt loam field soil was dried to 13 per cent moisture and maintained thus until 0, 5, 10, and 15 days after planting. Then the soil was watered to good moisture in each case for an additional 14 days. Radicles showed in dry soil in 5 days, but there was no emergence of plumules above the surface of dry soil in 15 days. Green seedling weights from 100 seeds planted, pericarp removed from the crown, not protected by a fungicide, were for 0, 5, 10, and 15 days, respectively, 50, 35, 7, and 4 gm. Treated with Arasan 2 oz. per bu. the weights were 59, 54, 31, and 20. Weights from seeds with sound pericarps were respectively 67, 65, 49, and 44 gm. untreated, 71, 67, 56, and 50 treated. Stands were nearly perfect throughout except where weights were down to 7 and 4 gm. Two fungi generally isolated from the interior of kernels planted in dry soil 5 days or more were *Trichoderma* species and an unidentified fungus producing only round, thick-walled hyaline chlamydospores, 15–22 μ, and septate mycelium.

Loss of leaf area increases damage from Gibberella stalk rot. KEOHLER, BENJAMIN. It has been known that loss of leaf area increases the susceptibility of corn plants to damage by Diplodia stalk rot. A similar effect has now been demonstrated for *Gibberella zeae*. Four single crosses were

used in 1952, all of them rated from previous experience as susceptible to Gibberella stalk rot. Inoculations were made about 15 days after tasselling, using toothpick cultures prepared from a *Gibberella* strain previously selected for pathogenicity. Cutting off the outer half of all leaves at the time of inoculation did not increase the extent of internode rotted by either *Gibberella* or *Diplodia* as long as stalks were green. *Gibberella* averaged 0.9 internode rotted against 1.9 for *Diplodia* in the same hybrids 4 weeks after inoculation. Technique used did not give results equivalent to natural severe Gibberella rot. Leaf clipping did promote premature death of plants and stalk breaking, the increase being least in the checks, significantly higher in the *Gibberella* inoculations, and highest in the *Diplodia* inoculations. A striking result was the development of natural Gibberella rot infections at above-ground nodes in 75 per cent of the plants with clipped leaves in contrast to such infections in only 17.3 per cent of plants with normal leaves.

The effect of KH_2PO_4 on germination of chlamydospores from Ustilago zeae (Beckm.) Ung. KOMMEDAHL, THOR. Chlamydospores of *Ustilago zeae* from a single gall were spread on Czapek's agar and stored at 3° C. for 30 days, then incubated at 27° C. from 24 to 28 hours when the germinated spores were counted. Both $KH_2P^{31}O_4$ and $KH_2P^{32}O_4$ were used in Czapek's medium. To obtain initial specific activities of 0.025, 0.05, 0.1, and 0.2 μc per ml., KH_2PO_4 at 1 g./l. proved sufficient; however, to attain initial levels of 5, 10, 20, and 40 μc/ml. of medium, 4, 8, 16, and 32 g., respectively, of $KH_2P^{32}O_4$ were needed per liter of medium. Any significant effect on numbers of spores germinated was that from KH_2PO_4 as such, and not from P^{32}, for each had a similar effect. Where 73 per cent of 7365 spores germinated on "Czapek's" (KH_2PO_4 at 1 g./l.), 44 per cent of 2806 spores germinated on that medium containing KH_2PO_4 at 32 g./l. With $KH_2P^{32}O_4$ in "Czapek's," 72 to 75 per cent of the spores at the 4 lowest specific activities (ca 2500 spores counted per level) germinated; whereas at the highest levels (32 g./l. at 40 μc/ml.), 36 per cent of 2568 spores germinated. Intermediate values obtained with intermediate levels.—This work was supported by the Atomic Energy Commission.

Host parasite relation in winter crown rot of alfalfa. LEBEAU, J. B., AND J. G. DICKSON. Winter crown rot of alfalfa and other forage plants, caused by a virulent unidentified low-temperature basidiomycete, attacks the crown area of the plants beneath the snow in the early spring. The mode of entry and development of the pathogen in the crown tissues of alfalfa plants inoculated and incubated at 2–4° C. was investigated. The fungus invaded the host either at the apex or at the base of the crown bud. Large masses of mycelium formed beneath the bud scales and then penetrated through the parenchymatous tissue into the vascular elements of the bud scales. The mycelium spread through the vascular system to the crown and shoot tissues. The mycelium was both inter- and intra-cellular and penetrated the cell walls without the formation of appressoria or special structures. No defensive response of the cells of the parenchyma or cambium of the host tissue of active or dormant alfalfa plants was evident. The production of HCN by the fungus growing on crown tissue and other media has been demonstrated. The quantities of HCN recovered from the crown tissues during progressive stages of disease development indicated that the fungus produced HCN in sufficient concentrations to kill the bud tissues prior to invasion.

Acidic buffer sprays and the control of early blight on tomato leaves. LEBEN, CURT. From 53 to 70 per cent disease control was obtained with the buffer-system H_3PO_4 + KH_2PO_4 at a concentration of 0.01 M and pH 3 in greenhouse experiments using the tomato early blight protectant test. Two other acidic buffer-systems, H_3PO_4 + NaH_2PO_4 (0.01 M, pH 3) and H_2SO_4 + Na_2SO_4 (0.05 M, pH 3), were also effective. When the phosphate buffers and the Na_2SO_4 solution were tried at pH 6, no control was obtained. After an 18 to 24 hour period at 26° C. growth of spore germ tubes of the disease inciting fungus, *Alternaria solani*, was inhibited to varying degrees in drops of 0.01 M phosphate buffer solution and on potato agar having pH values below 5.1. These pH levels were likewise inhibitory when the fungus was streak-seeded on the agar medium and macroscopic growth observed at 60 hours. Similar streak tests showed that a number of fungal phytopathogens were inhibited by media at pH 4.1 and below. Data suggest that special attention should be given to pH and buffering capacity in the study of toxicant materials.

A method of estimating numbers of Helminthosporium sativum spores in soil. LEDINGHAM, R. J., AND P. M. SIMMONDS. A technique which appears useful in assessing the population of conidia of *H. sativum* and perhaps other species of fungi in soil has been developed. Ten or 20 grams of sieved soil are placed in a 75-ml. test tube and shaken with about 50 ml. of water. Four ml. of a medium weight mineral oil are added, followed by vigorous agitation. On standing, the soil particles settle and the oil rises to the top. With sandy-loam soils about a half hour is required for settling. Between the water and oil layers is a temporary emulsion of water droplets in oil. The spores are found in the water droplets of this emulsion. By means of a fine pipette, aliquots of the emulsion are transferred to a microscope slide for examination. The average number of spores per aliquot related to the estimated total volume of emulsion and the weight of soil used gives an indication of the spore population.

The effect of a metabolic product on its subsequent production. LEGATOR, MARVIN, DAVID GOTTLIEB, AND VIRGINIA GALLICCHIO. The synthesis of chloramphenicol by *Streptomyces venezuelae* is limited by the concentration of this metabolite initially in the medium. The concentration that entirely prevents synthesis is the amount which is normally produced by the streptomycete in a medium containing no antibiotic. When lesser amounts are added, chloramphenicol is synthesized, but only until the limiting concentration is reached. These effects are not due to a toxic action of the antibiotic on the actinomycete, for *S. venezuelae* grew as well in the presence of 100 μg. chloramphenicol per ml. of medium as in its absence. No permanent deleterious effect on the antibiotic producing capacities of the organism result from the exposure to high concentrations of chloramphenicol. The mechanism is not an equilibrium between intracellular and extracellular concentrations of the antibiotic. The inhibiting effect of a metabolic product on its subsequent production might be a unique property of chloramphenicol. When terramycin is added to the medium before inoculation, the final concentration of terramycin is equal to the sum of the initital concentration and the quantity which would have been produced in its absence.

Antimycin A produced by a new isolate of Streptomyces. LOCKWOOD, J. L., AND G. W. KEITT. A crystalline antifungal antibiotic was isolated from the culture broth of *Streptomyces* sp., isolate 102. Melting points and agar-streak tests indicated that the crystalline product is identical with antimycin A, an antibiotic previously reported to be produced by a different organism, *Streptomyces* sp., isolate 35. Paper chromatograms, however, showed that antimycin A-102 (produced by isolate 102) and antimycin A-35 (produced by isolate 35) contained different proportions of 4 common components, which were named antimycins A_1, A_2, A_3, and A_4. Partly purified preparations of antimycin A-102 differed from similar preparations of antimycin A-35 in several respects, including toxicity to bacteria. Aqueous suspensions of partly purified antimycin A-102 gave average ED50 and ED95 values of 23 and 290 μg. per ml., respectively, in greenhouse tomato early blight protectant tests. Fifty-five per cent of the active material in similar preparations was retained on tomato foliage after washing with 2.5 cm. of simulated rain, while 70 per cent remained on unwashed foliage after 4 days. Concentrations of 2.8 mg. per ml. were nonphytotoxic to several species of plants. In preliminary seed treatment tests the partly purified antibiotic was as effective as Ceresan M in controlling the *Helminthosporium* seedling blight of oats (*H. vic-*

toriae).

Marasmius stenophyllus on abaca (Musa textilis) in Central America. LOEGERING, WILLIAM Q. *Marasmius stenophyllus* causes a rot of sheaths of abaca, the "Manila hemp" plant, that results in reduced production and quality of fiber. It occurs wherever abaca is grown in Central America and is reported from the Philippine Islands. Pathogenicity has been proved by inoculation experiments. The common abaca varieties in Central America are all susceptible though slight differences were observed in the amount of discoloration of tissues beyond the area of mycelial extension. Other species of *Musa* tested are somewhat less susceptible. Species of *Heliconia*, also in the Musaceae, are highly resistant. The disease is more severe on abaca subjected to unfavorable conditions though it can be found on plants growing under the best conditions. Fruiting bodies of *M. stenophyllus* are common on severely rotted plants and have been produced in culture by some isolates of the pathogen on sterilized pieces of abaca sheath. The principal means of spread is by direct contact between infected plant parts and healthy plants though occasionally spores from fruiting bodies may be a factor in dissemination. Incidence of the disease can be reduced by sanitation in harvesting procedures and losses can be reduced by harvesting infected plants before extensive deterioration has taken place.

Preliminary studies on an antibiotic produced by a species of Penicillium. LUKE, HERBERT H. AND LEANDER F. JOHNSON. A culture of *Penicillium* highly antagonistic to *Pythium arrhenomanes* was isolated from soil near Baton Rouge, Louisiana. Significant control of Pythium root rot of corn was obtained when this isolate was incorporated in soil, previously sterilized and artificially infested with *Pythium*. In agar culture the *Penicillium* isolate inhibited a number of plants parasitic fungi, including species of *Rhizoctonia, Helminthosporium, Fusarium, Colletotrichum, Sclerotium, Sclerotinia, Alternaria, Diplodina, Dendrophoma, Ascochyta*, and others. It did not inhibit the growth of *Pyrenochaeta terrestris, Botrytis cinerea*, or one isolate of *Rhizoctonia*. In similar tests with bacteria this isolate inhibited the growth of 9 gram-positive types and 7 gram-negative types. It was not effective against *Pseudomonas fluorescens* which is gram-negative. Methods for producing the antibiotic in liquid culture have been developed and preliminary attempts to isolate it have been made. No activity was lost when culture filtrates containing the antibiotic were heated at 100° C. for 40 minutes.

Relationships of naturally occurring strains of potato virus A. MACLACHLAN, D. S., AND R. H. LARSON. Systemic symptoms of virus A were observed on mechanically and *Myzus persicae* inoculated plants of *Nicandra physaloides* after 8 days at 24° C. *Lycium halimifolium, L. rhombifolium*, and *L. barbarum* produced local lesions as a result of mechanical inoculation, whereas *Lycopersicon pimpinellifolium* responded with a systemic necrosis. Three strains of virus A from 138 single tuber isolates of Green Mountain and Triumph were differentiated on the basis of their reaction on *N. physaloides*. The mildly virulent strain protected this host against the more virulent strains. There were distinct differences in the physical properties of the 3 strains. The varieties Kennebec, Katahdin, and Sebago did not become top necrotic when graft-inoculated with virus -A-infected scions from potato seedling 41956, and the virus was not recovered. The most virulent strain of virus A did not always incite the most severe disease on different clonal lines of Green Mountain. *M. persicae* proved to be an efficient vector, and virus A was found to be of the nonpersistent type. When starved *M. persicae* were used, the acquisition and inoculation thresholds were both 15 seconds. With feeding periods of 5 and 10 minutes, 2 consecutive plants became infected.

The utility of flower tissues for making inocula for difficult virus isolations. MCWHORTER, FRANK P. The abundant cytological evidence of virus in the tissues of petals of some flowers indicates high concentration of some viruses. Flower tissues may contain less material that inhibits transfer of virus than leaf tissues; also they are more easily triturated into effective inocula. Two virus-plant associations have been investigated to determine the relative value of flower inocula: 1) cucumber mosaic virus from the white streak disease of gladiolus, and 2) viruses from the latent virus complex of cherry. Inocula prepared from the flowers of gladiolus were only 4 times as effective as those prepared from corresponding leaves. Inocula prepared from flowers of cherry were, however, spectacularly superior to those prepared from leaves. Thus, when twin primary leaves of cowpea, *Vigna sinensis*, were inoculated with material from different source cherry trees, the local lesions induced ranged from 16 for flower inoculum and 1 for corresponding cherry leaf, to 175 for flower and none for leaf. In 1 series where test-plants were inoculated separately with flower and leaf inocula, the flower induced 440 lesions and the leaf induced none. In no series where good transfer was obtained did the transfer from leaves approach that from flowers.

Reduction in yield of corn due to stalk rot. MICHAELSON, MERLE E. AND J. J. CHRISTENSEN. Experiments were made during 1950 and through 1952 to determine the reduction in yield of corn due to stalk rot when infection occurs in specific parts of the stalk. Two varieties of corn were inoculated by the toothpick method with *Diplodia zeae* and *Gibberella zeae* in the second internode above the ground, in the ear shank, in the internode below the shank, and in various combinations of these 3 loci. The reduction in yield varied with the variety, the pathogen, the number of infections per stalk, and the season. In general, reduction in yield was greater in Minhybrid 504 than in Minhybrid 607; *D. zeae* caused greater reduction than *G. zeae*. A single infection with a given pathogen caused approximately the same reduction in yield regardless of the location of the rot in the stalk. The mean reduction in yield for the 3 years from a single infection was 9.7 per cent for *D. zeae* and 6.8 for *G. zeae*. With multiple inoculations per stalk reduction was increased to 12.5 per cent for *D. zeae* and 11.4 per cent for *G. zeae*. The average reduction in yield, varieties and pathogens combined, in 1950 was 13.2 per cent; in 1951, 7.4 per cent; and in 1952, 10.5 per cent.

The reaction of tomato varieties to various strains of alfalfa mosaic virus. MILBRATH, J. A. AND F. P. MCWHORTER. Great variations in susceptibility and symptomatology were observed following inoculation of 23 isolates of alfalfa mosaic virus into tomato. Some strains of the virus readily invaded every plant inoculated causing early and severe symptoms while others were transmitted to only a few plants. All strains tested gave distinctive symptoms on the varieties Marglobe, Stokesdale, Pritchard, Valiant, and Wasatch. Most strains induced black necrotic local lesions followed by systemic invasion causing necrosis in tip foliage closely resembling symptoms due to various strains of spotted wilt virus. Rapid invasion of small plants caused complete collapse and death. Plants usually withstood the initial shock reaction of the virus invasion and survived for some time. Some strains caused the death of the growing terminals, but the older leaves remained green and new lateral growth developed. Other strains caused systemic necrosis, but the terminals were not killed; eventually the older leaves turned yellow and withered, thus resembling symptoms of Fusarium wilt. Some strains that cause little or no necrosis produced a prominent leaf chlorosis with a strong green veinbanding pattern. The tomato is a good indicator plant for alfalfa mosaic virus and segregates various strains into distinct groups.

Transmission of components of the stonefruit latent virus complex to cowpea and cucumber from cherry flower petals. MILBRATH, J. A. Mechanical transmission of stonefruit viruses has been difficult. One virus component of the ringspot complex has been transmitted to cucumber by using young tip leaves as inoculum, but successful transfers have been low and erratic. Virus inhibitors or inactivators in cherry leaves are easily demonstrated, and probably play an important role in preventing mechanical transmis-

sion. The demonstration by McWhorter that tulip and gladiolus flowers contain virus and offer a more suitable source of inoculum than leaf tissues suggested similar procedures for stonefruit viruses. When inocula from tip leaves and from flower petals of Montmorency sour cherries suspected of containing latent viruses were compared on cowpea and cucumber, 2 distinct reactions occurred. The leaf inoculum gave the expected reaction associated with the ringspot complex on cucumber, but no reaction on cowpea. The inoculum from the flower petal gave numerous local necrotic lesions on the primary leaves of cowpea and chlorotic lesions with a white center on the cucumber cotyledons. Occasionally cucumbers developed symptoms described for ringspot virus, but usually the virus obtained from the petals gave a distinct reaction suggesting a different virus or strain.

Interaction between fungus spores and toxicants in simultaneous and consecutive exposures. Miller, Lawrence P., S. E. A. McCallan, and Richard M. Weed. When spores of a number of species of fungi are exposed to dilute solutions of such fungitoxicants as 2-heptadecyl-2-imidazoline, silver, or cerium, large quantities on a spore weight basis, are taken out of solution very rapidly. Extensive experiments in which spores were treated either with mixtures of toxicants or exposed alternately to different toxicants have indicated a marked lack of effect of one toxicant upon another. Even when saturated with one fungicide a second one was rapidly taken up. These results suggest that sites of accumulation for the various toxicants may not be the same. Using cerous sulfate, labeled with Ce^{144} and S^{35} it was found that uptake of cerium was quite independent of that of the sulfate ion. The uptake of the imidazoline was considerably retarded by small quantities of Tergitol-7. Drastic procedures were necessary to remove the toxicants from spores after uptake even when the spores had only been in brief contact with the fungicidal solutions. Preliminary experiments with spores of *Neurospora sitophila* indicate that a small percentage of the C^{14} in the ring labeled imidazoline appears in the respiratory CO_2. These studies were carried out in cooperation with the Atomic Energy Commission.

An apparently new virus of tomatoes in Illinois. Miller, Patrick M. A virus (designated V-52-1) with some properties of common tobacco mosaic virus (TMV) has been identified as the cause of a new virus disease of tomatoes in Illinois. Clearing of veins, occasional necrosis of leaf areas, and interveinal chlorosis with veinal areas remaining green characterize the disease under field conditions. In greenhouse plants only systemic mottling occurs. V-52-1 and TMV are similar in the following characteristics: systemic mottling in tomatoes under greenhouse conditions, and in *Nicotiana tabacum* variety Turkish; necrotic local lesions in *N. glutinosa*, *Datura stramonium*, and *Solanum melogena*; dilution end point of 10^{-6}; inactivation at pH 11.0 for 10 minutes; inactivation and precipitin reaction from V-52-1 guinea pig antiserum; and morphology of 15×280 mμ rods. V-52-1 differs from TMV in the following characteristics: necrotic local lesions on *Vigra sinensis* variety Black Eye (large); absence of necrotic lesions on *Phaseolus vulgaris* varieties Scotia, Pinto, Early Golden Cluster, Idaho Refugee, and Great Northern; thermal inactivation at 70° C. for 10 minutes; and instability during storage at 2° C. and freezing.

The influence of the ring spot virus on growth of one-year-old sweet cherry nursery trees. Millikan, D. F. Field observations on 1-year-old nursery trees propagated from infected and ring spot-free sources indicated that ring spot virus is a factor in growth inhibition of sweet cherry. Measurements made in 1952 on Lambert propagated from nursery-run and ring spot-free sources indicated an average total growth of 97.63 and 117.73 in., respectively, with the greatest growth inhibition occurring during the middle part of the growing season. In 1952, Lambert propagated from a Barnard 1–12 infected source and a ring spot-free source averaged 80.62 and 140.5 in., while the same variety propagated from a Goldman 2–1 infected source averaged 57.44 in. compared with 159.33 in. for trees of the same variety propagated from a ring spot-free source. The Hardy Giant variety propagated from a C-297 infected source averaged 75.0 in. contrasting with 112.86 in. for the control trees propagated from ring spot-free sources. Greatest difference for both varieties appeared to be during the June-July period of the growing season. June inoculations of S-8 ring spot virus on ring spot-free nursery trees of both varieties caused a pronounced growth reduction. This effect remained apparent throughout the season on the Lambert variety while the Hardy Giant variety appeared to recover 2 weeks following inoculation.

Movement of Streptomycin in Bean Plants. Mitchell, John W., William J. Zaumeyer and William H. Preston, Jr. Antibiotic activity was detected in juice from leaves of bean plants following application of streptomycin sulfate to their stems. The translocated antibiotic appeared to be streptomycin since a streptomycin-dependent strain of *Escherichia coli* grew on nutrient agar impregnated with the translocated material. Twenty-five micrograms of streptomycin on stems reduced severity of halo blight symptoms which developed on nearby leaves inoculated with the organism; 50 micrograms arrested symptom development, and 100 micrograms completely prevented symptom development. Translocation of streptomycin was upward through the stems. Movement of the antibiotic from the proximal toward the distal portions of leaves was demonstrated but not movement in the reverse direction. Translocation of streptomycin from leaves to stems and fruits could not be demonstrated. The concentration of streptomycin in juice from leaves near a localized stem application of streptomycin sulfate reached a measurable level within a few hours after treatment, increased during the next 3 days, then gradually decreased to a relatively low level. Absorption of streptomycin from soil treated with relatively large amounts could not be demonstrated. The halo blight organism was eradicated by dipping artificially inoculated plants into a 0.025 percent solution of streptomycin shortly after the appearance of initial symptoms. Control was much less effective if treatment was delayed several days.

Synchytrium endobioticum on wild potatoes in Mexico. Niederhauser, John S. *Synchytrium endobioticum* has been collected at 3 different locations in central Mexico on *Solanum stoloniferum*, *S. vallis-mexici*, and Solanum sp. In at least 2 of these locations the possibility of contamination from introduced potatoes is very remote. This suggests that *Sychytrium endobioticum* is endemic in the western hemisphere. Attempts to inoculate the susceptible Up-to-Date variety with the Mexican material were unsuccessful.

Races of Phytophthora infestans prevalent in central Mexico. Niederhauser, John S. and Javier Cervantes. Several isolates of *Phytophthora infestans* from the high valleys in Central Mexico will severely attack all the differentials developed by Drs. Reddick, Peterson, and Mills (race BCD in their classification), as well as all the available differentials (R_1, R_2, R_3, R_4) developed in Scotland by Dr. William Black. Some cultures can produce good infection on all clones of *Solanum demissum* that have been tested to date. Direct evidence has been found for the occurrence of a fourth gene for late blight resistance in *Solanum demissum*.

Liquid concentrates versus dusts for in-the-row treatments against seedling diseases and insect pests, with special reference to onion smut and maggot. Newhall, A. G., W. A Rawlins, J. L. Brann and W. W. Gunkel. New equipment designed to blow 10 to 30 lb. per acre of a fungicide-insecticide-Pyrax dust into the furrow as seed is dropped resulted in good control of onion smut and of maggot on 6 farms. Fungicides compared at 3 concentrations were Tersan, Arasan, and Orthocide. To each Tersan-Pyrax combination was added 1 of 3 quantities of Aldrin, Dieldrin, Heptachlor, or Parathion wettable powder. In applying 15 lb. per acre of these mixtures, 2, 3, or 4 lb. of the fungicide was actually used plus 1¼, 2½, or 5 lb. of Aldrin 20 per cent W. P.; or ½, 1, or 2 lb. of Dieldrin

25 percent W. P.; or 1, 2, or 3 lb. of Heptachlor 25 percent W. P.; or approximately 1¾, 3¼, or 6¾ lb. of Parathion 15 percent W. P., respectively. A specially designed gas-engine driven blower feeding dust to 4 flexible tubes assured even application to 4 rows at a time, instead of 2 as in previous years. Seedling smut was reduced on 4 farms from 25, 25, 37, and 50 per cent in the checks to 2, 1.2, .8, and 4.3 per cent respectively. With other equipment, concentrated formaldehyde-liquid insecticide combinations, sprayed into the furrow, compared favorably with the regular drip and the dry pelleting methods of smut and maggot control. No fungicide-insecticide incompatibility was encountered.

Physiological changes induced in peach foliage by the Western X disease virus. O'REILLY, H. J. In peach foliage infected with the Western X disease virus, respiration rates, starch content, and nitrogen content were determined. Factors affecting respiration of healthy leaves were studied to evaluate respiration of diseased leaves. Respiration of healthy leaf tissue determined on a fresh weight basis was greatest at the terminal of elongating stems and decreased as terminal buds matured. Respiration in all leaves decreased from terminal to base of current season growth. In comparing leaves from separate stems, comparable stages of leaf and terminal bud maturity had to be considered. Respiration of healthy tissue determined in the afternoon exceeded that of the morning. Diseased leaves respired more rapidly than healthy leaves, increasing in rate as symptoms became more severe. The increase occurred when determined on a fresh weight basis and on a total nitrogen basis. Foliar starch accumulation increased with disease severity, but total nitrogen content was lower than in healthy foliage and did not vary as the disease advanced. The data on respiration, starch accummulation, and decrease in nitrogen all add to the evidence that the Western X disease virus should be classified with the yellows group of viruses.

Occurrence of a Fusarium wilt of cucumber in Florida. OWEN, JOHN H. A wilt of cucumber was first observed in Sumter County, Florida, in 1929 where cucumbers were grown continuously on the same soil for several years. Percentage of infection ranged from 15–100 per cent in some fields. A *Fusarium* sp. was isolated from the stems and roots of infected plants. All stages of plants became infected, with the young seedlings being most susceptible. The fungus first invades and discolors the cortical tissues of the tap root. This is followed by their gradual breakdown and by a brown discoloration of the vascular tissue of the stem. Infected plants wilt and the leaves die progressively from the crown. Twenty-three varieties of cucumber planted in inoculated soil showed 16–100 per cent infection. The fungus caused from 7–14 per cent infection of watermelon and 100 per cent infection of cantaloupe. Squash, pumpkin, and gherkin were not infected. Cross inoculation with *Fusarium oxysporum* f. *niveum* from watermelon showed no infection of cucumber, cantaloupe, squash, pumpkin or gherkin. One-hundred per cent infection was obtained on some varieties of watermelon.

Studies on the infection type produced on Kubanka wheat by certain physiologic races of Puccinia graminis tritici. PAPAVIZAS, GEORGE C. Kubanka wheat normally has a mesothetic reaction (type X) to races 32, 36, 49, and 139 of *Puccinia graminis tritici.* In some environments the mesothetic reaction may change to susceptibility and in other environments to resistance, because the infection type X is sensitive to temperature and light. Kubanka seedlings, inoculated with each race and kept in moist chambers for 24 hours, were placed in different environments on the second day after inoculation. Race 36 produced type X in only a single environment, 70–75° F. and normal sunlight of 8 hours' duration. Races 32, 49, and 139 produced type X at 80–85° F., 70–75° F. and 64–67° F. with normal sunlight; likewise, they produced type X at 70–75° F. with normal sunlight plus supplemental artificial light at night and at 70–75° F. with reduced light for 6, 12, and 18 days. Kubanka was resistant to the 4 races at 64–67° F. if light intensity was reduced to approximately ½ normal sunlight. The variety was susceptible to the 4 races at 80–85° F. under normal sunlight supplemented by artificial light at night.

Green ring mottle virus reduces quality of sour cherry fruit. PARKER, K. G. AND E. J. KLOS. Inoculations to Montmorency from the same variety with discolored, pitted fruit and from English Morello with small, late-maturing fruit caused fruit symptoms typical of those on the Montmorency source trees and leaf symptoms typical of green ring mottle. The fruit of affected trees of both varieties is off flavor. Inoculations to English Morello and to Napoleon sweet cherry did not cause definitely recognizable symptoms in 2 years. Green ring mottle symptoms are associated with the fruit symptoms on Montmorency in the orchard, but no leaf symptoms have been observed on the affected English Morello trees. Field surveys indicate orchard spread occurs; the disease appears to be of considerable importance, particularly on the English Morello variety.

Blossom and twig blight of low-bush blueberry. PELLETIER, E. NEIL AND M. T. HILBORN. Blossom and twig blight of low-bush blueberry is caused by *Botrytis cinerea* Pers. which infects developing buds, stems, and flowers if high humidity is maintained for at least 72 hours. There is a wide range of susceptibility within clones, and early chemical soil fertilization favors infection. A total of 41 fungicides and 12 antibiotics were tested for control. The phenyl mercuries, dithiocarbamates, and some heterocyclic nitrogen compounds were more effective than the inorganic metals, inorganic sulfurs, and antibiotics. In slide germination tests elemental sulfur and lime sulfur were the least, and phenyl mercuries the most, effective. Phenyl mercuries, some inorganic coppers, and the mixed glyoxalidines (341) were phytotoxic in greenhouse tests. Of the antibiotics, rimocidin, thiolutin, and streptomycin sulfate were very inhibitory in slide germination tests but failed to control the disease in greenhouse tests. Vancide 51 Manzate, ferbam, ziram, and captan were most effective as fungicides in greenhouse tests, all giving complete control. In field tests ferbam gave 100 per cent, ziram 69 per cent, and Manzate 33 per cent control respectively. Phygon, mediocre in greenhouse tests, also gave complete control in field tests.

Amino acids as nitrogen sources for Venturia inaequalis. PELLETIER, R. L. AND G. W. KEITT. *Venturia inaequalis* was grown in shake-flask cultures on 2 basal synthetic media to which were added singly 23 nitrogen sources. Both basal media contained glucose, salts, trace elements, and Tween 80, but 1 had thiamine only, and the other had 10 vitamins, nucleic acid and nucleic acid hydrolysate. The weight of mycelium produced was taken as a measure of growth. The incubations periods were 7, 14, and 21 days. The nitrogen sources were used at concentrations of 0.05, 0.1 and 0.2 gram of nitrogen per liter. There were 3 replicates. The data were analyzed statistically. The growth responses of the fungus varied with the nitrogen sources, the incubation periods, the basal media and the concentrations. Moreover, there were significant interactions between these different treatments. However, it is possible to group the nitrogen sources into 3 classes which are independent of the experimental conditions used: *"good"* sources of nitrogen, L-proline, DL-alanine, L-tyrosine, L-glutamic acid, glycine, L-arginine, casein hydrolysate, and a mixture of 19 amino acids; *"fair"* sources of nitrogen, DL-leucine, DL-isoleucine, DL-threonine, DL-valine, DL-phenylalanine, DL-serine, DL-aspartic acid, L-asparagine, and ammonium nitrate: *"poor"* sources of nitrogen, L-cystine, DL-tryptophane, DL-methionine, L-hydroxyproline, L-lysine, and L-histidine.

The relation of Cladosporium cucumerinum to resistant and susceptible cucumber tissues. PIERSON, C. F. *Cladosporium cucumerinum* Ell. and Arth., the incitant of cucumber scab, penetrates cucumber tissues directly from well defined appressoria 48 hours after inoculation, when the plants are held at 17° C. in a saturated atmosphere. The

fungus is initially intercellular in both resistant and susceptible hosts, but it shifts to an intracellular habit in the susceptible host 96 hours after inoculation. Coagulation of cytoplasm and changes in host cell staining reactions are associated with this shift in the habit of the fungus. Within 120 hours after inoculation sunken lesions are formed by the necrotic collapse and lysis of host cells. These processes contribute to the formation of cavities beneath the sunken lesions of fruits, stems, and petioles and to a shot-holing of the leaves. In the resistant host the fungus seldom becomes intracellular and the progress of the disease is arrested by host reactions which are associated with cell wall thickening and necrosis. Sunken lesions are rare, while cavities and shot-holing are absent.

Characteristics of germinating chlamydospores of loose smut of wheat and of barley. Popp, W. Chlamydospores of 5 races of *Ustilago tritici* and 5 races of *U. nuda* were studied. Within a period of 24 hours on Difco potato-dextrose agar at 20°C. the chlamydospores of *U. tritici* germinated to form a 4–8 celled promycelium, each cell of which gave rise to one or more branching and septate hyphae containing haploid cells. The germinating spores of *U. nuda* were characterized by a relatively short promycelium of not more than 4 cells which fused in pairs and formed septate and sparsely branched hyphae containing diploid and haploid cells. The type of structures produced by the germinating spores are clearly evident after flooding them with a drop of cotton blue in lacto-phenol. On water agar both smuts were characterized by the fusion of promycelial cells in pairs and the production of single celled diploid hyphae. On potato-dextrose agar with reduced nutrients the germinating spores of both smuts tended to form structures varying from those produced on water agar to those produced on Difco potato-dextrose agar. The promycelia of *U. tritici* and *U. nuda* had an average length of 45 μ and 22 μ respectively. Thus, from the results of the study of 5 races of each smut, there is evidence that *U. tritici* and *U. nuda* can be differentiated on the basis of the morphology of germinating spores.

Effects of air and soil temperature on the multiplication of turnip virus 1 in certain species of Nicotiana. Pound, Glenn S. and Lewis G. Weathers. Three strains of turnip virus 1 (Cabbage A, cabbage black ring, horseradish HR1) developed systemically in *Nicotiana glutinosa* more rapidly and in greater concentration at an air temperature of 16°C. than at 28°. A concentration gradient occurred at temperatures of 16, 20, 24, 28°. Systemic movement and multiplication at 28° were practically nil. These air temperature reactions were completely reversible with reversal in temperature. In inoculated leaves, however, rate of multiplication increased with increase in air temperature. Air temperatures had little effect on ultimate virus concentration of the 2 cabbage strains in *N. multivalvis*. In *N. rustica* the cabbage A strain multiplied most rapidly and in greatest concentration at 20°. With the 2 cabbage strains, soil temperature affected multiplication in *N. glutinosa* in the same direction as air temperature although to a lesser degree; i.e. virus concentration increased from 28° to 16° soil temperature. Increasing the soil temperature at 16° air temperature and reducing it at 28° air temperature enhanced multiplication over conditions where air and soil temperatures were the same. Soil temperature effects were also reversible. Growing the plants at different levels of nutrition at 16° and 28° air temperature did not alter the symptoms or virus multiplication in *N. glutinosa*.

An evaluation of chemical treatment for the control of Botrytis cinerea on Strawberry. Powell, Dwight. Fungicide experiments have indicated promising results for gray mold control but small, replicated plots have not been satisfactory for a full season evaluation. A 2-acre patch of Blakemore was treated with 50 per cent Captan 4 times at 7-day intervals starting with the first bloom at the rate of 6 lbs. per acre per treatment. Prior to the first picking adjacent untreated rows showed 11 per cent mature fruit rot as compared to 1 per cent in the treated area. A wide variation of intensity existed within the treated (0–5 per cent) and untreated area (8–16 per cent). There was a gradation of infection from the area next to the untreated rows to the center of the treated area (0–5 per cent). It is evident that the dissemination of gray mold is so rapid that chemical treatment is not too satisfactory when untreated patches exist in a treated area.

Cultural studies of Armillaria mellea. Raabe, Robert D. A wide range of variability in cultural characters has been observed in *Armillaria mellea*, not only when isolated from different hosts but occasionally when isolated from different portions of the same host. In addition, 56 monospore isolates from a single sporophore have shown extremes in cultural variation. The cultures vary in color and in the amount and rate of mycelial growth and rhizomorph production. Although pH and nutrient level affect the rate of growth and the amount of rhizomorph production, light and the availability of moisture are found to have considerable effect upon these characters of the fungus.

Sensitivity of Fusarium vasinfectum to antagonistic microorganisms in the soil. Ram, C. S. Venkata. *Fusarium vasinfectum* is somewhat sensitive to microbial antagonism, and its mycelium rapidly decomposes in soil. This organism, however, can survive and even grow on an organic substrate in the presence of antagonistic organisms. Autoclaved soil in flasks was artificially infested with soil isolates of bacteria, actinomycetes, and fungi—excluding Fusaria—and incubated for 3 weeks. Two pieces of autoclaved cotton stem, 9 cm. long, were thrust into the soil in each flask and incubated for varying times prior to inoculation with *F. vasinfectum*. Agar discs on which *F. vasinfectum* was growing were placed on the exposed end of the stem, in the soil beneath the stem, and on the soil surface adjacent to the stem. The spread of the fungus within the stem during various incubation periods was determined by cutting 1.5 cm. lengths of the stem and plating them *seriatim*. The *F. vasinfectum* colonized both fresh plant stem pieces as well as those colonized with other soil microorganisms. This fungus grew within the organic substrate regardless of the presence of microbial antagonists; however, the rate and amount of growth decreased with increase in the antagonistic organisms in the organic substrate.

The breaking of tobacco mosaic virus using a new freeze drying method. Rice, Robert V., Paul Kaesberg, and Mark A. Stahmann. During the development of a spray freeze drying method of preparing specimens for electron microscopy, tobacco mosaic virus (TMV) rods were observed broken into smaller lengths when sprayed onto collodion covered screens and dried at low temperatures. These broken pieces were found to be arranged linearly when viewed in the electron microscope indicating that they originated from individual particles. The size distribution of the small broken pieces show a sharp distribution about 400 A. The 3000 A virus particles break into a maximum of 8 pieces, the pieces lie in an approximate straight line relative to their long axis, and the sums of the lengths of the broken pieces are about the same as the length of the unbroken particles. The largest proportion of broken particles is obtained when solutions of TMV are sprayed onto collodion covered microscope screens mounted on metal blocks in contact with liquid air suggesting that breakage is associated with rapid freezing. The results suggest that the cleavage may be the result of contraction during the rapid freezing or drying and imply that the virus particles may be made up of smaller sub-units.

Relation of polyphenol oxidases to fungitoxicity. Rich, Saul and James G. Horsfall. A mycelial extract from *Stemphylium sarcinaeforme* contains polyphenol oxidase of the laccase type, which increases in activity when stored at 3°C. This extract was tested on 50 quinones and phenols which varied in their toxicity to *S. sarcinaeforme*. Those compounds oxidized by the enzyme were weakly toxic or non-toxic to the fungus. Compounds not oxidized by the enzyme were toxic, except for certain *para* substituted phenols. In addition, certain of the toxicants were found to be inhibitors of the enzyme. Some of these inhibitors

were protocatechuic acid, α-naphthol, β-naphthol, 2,5-dihydroxyterephthallic acid, 3,5-diiodotyrosine, and salicylic acid. Chloranilic acid, not oxidised itself, accelerated polyphenol oxidase activity when first mixed with a substrate, but slowed it when mixed with the enzyme prior to testing. Mycelial extracts of *Sclerotinia fructicola* gave polyphenol oxidase activity of the tyrosinase type. No consistent correlation existed between the toxicity of phenols and quinones to *S. fructicola* and their interaction with extracts from this fungus. Polyphenol oxidase is postulated to be an important terminal oxidase in *S. sarcinaeforme*, but not in *S. fructicola*.

The effect of root-knot nematodes (Meloidogyne spp.) on the expression of black shank resistance in tobacco. SASSER, J. N., H. R. POWERS, JR., AND G. B. LUCAS. Black-shank-resistant tobacco seedlings transplanted to pots of steamed soil were treated as follows: 1) no treatment, 2) inoculated with *Phytophthora paraasitica* var. *nicotianae*, 3) inoculated with single species of *Meloidogyne*, and 4) inoculated with both *P. parasitica* var. *nicotianae* and *Meloidogyne*. Seedlings in 1), 2), and 3) grew well, but those in 4) wilted within a week. One week later all plants growing in soil inoculated with both pathogens had black-shank symptoms and within 1 month all were dead. In other tests black-shank-resistant seedlings were transplanted to steamed soil inoculated with single species of *Meloidogyne*. These plants were allowed to grow 30 days and the soil was then inoculated with *P. parasitica* var. *nicotianae*. One week later some of the nematode infected plants growing in soil inoculated with *P. parasitica* var. *nicotianae* showed black-shank symptoms, whereas root-knot free plants of the same age, growing in soil inoculated with the black-shank fungus at the same time, showed no black-shank symptoms. Thirty days after inoculation, 78 per cent of nematode-infected plants had black shank. The root knot free plants inoculated with *P. parasitica* var. *nicotianae* showed no black shank symptoms. These results indicate that *Meloidogyne* altered the expression of black-shank resistance of the tobacco varieties used.

Effect of metabolic inhibitors on Fusarium resistance in tomato. SCHEFFER, R. P., AND S. S. GOTHOSKAR. Jefferson tomato plants, homozygous for single-gene resistance to *Fusarium oxysporum* f. *lycopersici*, when inoculated through cut ends of stems developed a shock phase of wilt from which they promptly recovered, while cuttings of susceptible Bonny Best showed the typical disease syndrome. Cut ends of plants were placed after inoculation in solutions of several respiration inhibitors. Inoculated, untreated cuttings and uninoculated, treated cuttings were used as controls. Inoculated Jefferson cuttings treated with 2, 4 dinitrophenol 10^{-5} M, thiourea 5×10^{-4} M and sodium fluoride 2×10^{-4} M developed disease similar to Bonny Best. At the concentrations indicated, the materials caused little or no inhibition of growth of the pathogen *in vitro*. Results with sodium bisulfite 10^{-3} to 10^{-4} M and sodium diethyl dithiocarbamate 10^{-3} to 10^{-4} M were inconclusive. Urethane 10^{-2} M, potassium cyanide 10^{-5} M, sodium azide 10^{-4} M, sodium malonate 10^{-2} M and sodium fluoroacetate 10^{-3} M failed to decrease resistance. Negative results are not necessarily significant, since some compounds may inhibit growth of the pathogen *in vivo*, or may not be absorbed by host cells. Data to date do not show that resistance is associated with any specific step in respiration, but do indicate that resistance may be supported by active metabolic reactions.

Microorganisms in seeds and plants of certified beans. SCHNATHORST, WILLIAM C. AND H. J. WALTERS. Isolations were made from bean varieties (Great Northern, U. I., Nos. 16 and 123; Pinto, U. I., Nos. 78 and 111), grown and certified in Idaho, by 6 methods: surface sterilized, macerated seeds; dissected and plated seeds; plated seeds; broth cultured seeds; serial dilution of macerated plant tissues from various portions of plants; and surface sterilized, dissected 4-in. portions of stems placed on potato-dextrose agar. Bacterial isolates were subjected to 24 determinative tests including inoculations to *Glycine Max*, *Melilotus officinalis*, *Phaseolus lunatus* var. *limensis*, *P. vulgaris*, and *Vigna sinensis* var. blackeye. Fungal pathogenicity was tested by growing the above plant species in jars of sterilized sand containing the isolate of the desired fungus. Four bacterial species existed in seeds only, 5 in seeds and plants, and 14 in and on plants only. The genus *Bacillus* was most prevalent. None of the bacteria were pathogenic. *Sporotricum* sp. and 1 strain of *Rhizoctonia solani* were found in vascular tissues; *Alternaria* sp., *Stemphylium* sp., and 3 strains of *Rhizoctonia solani* were found in seeds. *R. solani* and *Sporotricum* sp. were pathogenic.

An orange-colored bacterium comparable to Corynebacterium flaccumfaciens (Hedges) Dowson causing bean wilt. SCHUSTER, M. L. AND D. W. CHRISTIANSEN. Representative cull samples of field bean seed from western Nebraska included about 30 per cent orange-colored seed. This color was due to the presence of bacteria and was found in Great Northern and Pinto varieties. Seed infection detectable macroscopically was not commonly obtained from soil infestation or by inoculation of first or second nodes of the bean plant. Discolored seed similar to cull samples was obtained by inoculation of the upper nodes or by rubbing the bean pods with a bacterial suspension. Physiological and morphological tests indicated close similarities of the orange-colored bacterium to *Corynebacterium flaccumfaciens*. The orange-colored seed is an aid in differentiating this organism from the common blight bacterium, *Xanthomonas phaseoli*, which is common in western Nebraska. The orange-colored bacterium may be considered as a strain of *Corynebacterium flaccumfaciens*, the yellow-colored bean wilt organism.

Onion blast or leaf spotting caused by species of Botrytis. SEGALL, R. H. Onion leaf spotting and tip blighting, known in New York as blast and formerly believed to be a physiogenic disease, have been found to be caused by various species of *Botrytis* including *Botrytis Allii* Munn, *B. cinerea* Pers. ex Pr., *B. Tulipae* (Lib.) Lind., and *B. Paeoniae* Oud. In commercial onion fields *B. Allii* was found sporulating abundantly on dead onion foliage. These spores are the primary source of inoculum as no sporulation was found on the individual leaf spots. Typical symptoms were produced by artificially inoculating leaves with a spore suspension of these species. No evidence of cuticular or stomatal penetration by the germ tube or mycelium on naturally or artificially inoculated leaves has been observed. A minimum period of 22 hours of saturated humidity is necessary for spotting to occur. Leaves give off enough water due to transpiration to allow the spores to germinate and the spots to appear. In the greenhouse if conditions are not favorable for spore germination, the spores are able to cause spotting up to 6 days following inoculation. For the spotting to occur, it is necessary to have natural light or the artificial equivalent. In darkness only occasional spots develop.

Factors affecting germ tube development in Puccinia coronata Avenae. SHARP, E. L. AND F. G. SMITH. Zinc and gelatin dialysate in the proper pH range stimulated characteristic germ tube branching and the formation of appressoria, substomatal vesicles, infection hyphae, and haustorium mother cells by uredospores of P. coronata. Amino acids, nucsleotide derivatives, and growth factors could not be substituted for gelatin. However, the gelatin factor could be replaced by silicon, completely in the branching response and at least partly in the vesicle response. Ten p.p.m. of zinc, 2.5 to 5 p.p.m. of silicon, caused maximum stimulation of vesicle formation on water agar. On gelatin, vesicle formation was inhibited by 10 p.p.m. of silicon or more, and germination was inhibited by about 50 p.p.m. It appears that both zinc and silicon are necessary for branching and vesicle formation on artificial media and that silicon may be identical with the gelatin factor.

Physiologic specialization in Venturia inaequalis. SHAY, J. R. AND EDWIN B. WILLIAMS. About 30 clones representing 14 species of *Malus* have been found to be resistant to

all strains of *Venturia inaequalis* tested from Illinois, Indiana, and from Australia, England, Germany, Norway and Yugoslavia. In 1951, natural infections were found in South Dakota on the previously resistant *Malus baccata* varieties Alexis and Dolgo, and in Nova Scotia on the previously resistant ornamental variety Geneva (*M. niedzwetzkyana* o.p.). The host specialization of cultures from these 2 unusual infections have been compared for 2 seasons with that of the commonly occurring strains (Race 1) in artificial infection trials on all resistant sources. It was found that Race 2 (South Dakota collection) differs from Race 1 in its ability to attack the *M. baccata* varieties Dolgo, Alexis, and Bitter Crab as well as certain segregates of a Russian apple, R12740-7A. Race 3 (Nova Scotia collection) differs from Race 1 in its ability to attack Geneva. All the other resistance sources were resistant to the 2 new races.

Factors that influence Rhizoctonia solani to incite turf brown patch. SHURTLEFF, M. C. Rhizoctonia solani isolates, capable of causing severe foliage blight (brown patch) of turf grasses, varied greatly in their ability to incite crown necrosis, root rot, and damping-off. Penetration of grass leaves was either (1) primarily stomatal, (2) primarily cuticular, or (3) equally stomatal-cuticular, and characteristic for the isolate. Mowing wounds (0,1,3 days old) did not influence degree of foliar infection on 5 grass species of various ages. Mycelium survived in dry grass clippings up to 4 months. Sclerotia were viable after 25 successive germinations on water or potato-dextrose agars. Certain isolates formed sclerotia on grass blades and crowns 3 days after foliage wilt. Sclerotia germinated: 1) only at sustained humidities of 98 per cent or above regardless of isolate or age; 2) at temperatures of 15.6° to 37°C. During favorable meteorological conditions brown patch areas increased approximately 1 in. per day and spread was unchecked after applying a phenyl mercury solution. *R. solani* advanced from 1 to 2 cm. per day through soil in flats containing Astoria Colonial bent at greenhouse temperatures of 19° and 27°C. Fungus spread was most rapid at the higher temperature and at the highest of 5 seeding rates. Initial foliar infection occurred on leaves in contact with soil after which disease progressed up the plant.

Wind and blowing soil as factors in the spread of wheat streak-mosaic virus. SILL, W. H., JR. Using a wind tunnel, air was blown at 30 m.p.h. through diseased and healthy month-old greenhouse wheat plants, var. Westar, set alternately side by side so that lashing leaves would intermingle. Injury owing to abrasive action was visible after 6 minutes on both diseased and healthy plants. Eight per cent of 286 healthy plants developed mosaic symptoms in 3 trials comprising 8 separate runs. The addition of screened (.59 mm) silt loam soil blowing at the same wind velocity did not increase the percentage of plants infected nor did the use of the same soil artificially infested with a 1–20 inoculum of the virus. The infested soil blown through healthy plants that were not associated with diseased plants caused no infection. Healthy plants subjected to wind only or to wind plus noninfested soil also remained symptomless. The data indicate that a relatively low percentage of healthy plants may be infected during strong winds by direct abrasive contact with lashing leaves of diseased plants and that blowing soil is probably not a factor in the transmission of wheat streak-mosaic virus.

Infections of crown roots of wheat in relation to common rootrot injury. SIMMONDS, P. M. There are large areas in Saskatchewan where severe injury appears late in the season in wheat crops. The damage is shown by reduced yields and lower grades. Evidence from field and laboratory work has been obtained indicating the prime importance of common rootrot of the *Helminthosporium sativum* type in this trouble. Importance of healthy crown roots through the ripening period is known. Several fields of Thatcher wheat showing rootrot patches were selected for study. The study indicated a progressive increase in the percentage of diseased roots as the season advanced. For instance in one typical field on July 22, when the plants were well headed the number of severely infected crown roots on plants from a rootrot area was 47 per cent; at harvest time, August 23, there were 90 per cent infected roots and the average plant yield was 0.87 grams. The healthier plants from adjacent areas at harvest time showed only 5 per cent of the crown roots severely infected and an average plant yield of 1.52 grams. These results are quite typical of the fields studied. There appear to be certain factors which predispose the plants so that those individual plants bearing well established crown infections deteriorate rapidly.

The relationship of temperature and stage of growth to the crown rust reaction of certain varieties of oats. SIMONS, M. D. Two lots of oats, each including 11 varieties at 4 stages of growth (seedling, juvenile, boot and anthesis) were inoculated simultaneously with race 205 of crown rust (*Puccinia coronata* Cda. var. *avenae* Fraser & Ledingham). One lot was incubated at about 15° C. and the other at about 25° C. Some varieties, such as Markton, reacted similarly at both temperatures and at all stages of growth, while others differed. With few exceptions, varieties differing in reaction were more resistant at the low temperature and in the later stages of growth. On a given plant older leaves were more resistant than younger leaves. Mo. 0-205, completely susceptible in the seedling stage, showed a very high degree of mature plant resistance at both temperatures. At 15° C. Appler, Marion, and Cherokee, also susceptible in the seedling stage, exhibited moderate mature plant resistance. At 25° C. these varieties were susceptible at all stages of growth. Santa Fe, Bondvic, Clintafe and Clintland were uniformly, highly resistant at both temperatures and at all states of growth. Landhafer was likewise highly resistant at 15° C., but at 25 ° C. this resistance broke down sufficient at all stages to clearly differentiate Landhafer from the other highly resistant varieties.

Aphid transmission of the Wisconsin pea streak virus. SKOTLAND, C. B. Greenhouse experiments performed during the past 3 years have established the pea aphid, *Illinoia pisi* (Kalt.), as a vector of the Wisconsin pea streak virus. A low percentage (approximately 5 per cent) of trials were successful, indicating that the pea aphid is apparently an inefficient vector of this virus. Acquisition threshold was found to be as low as 15 seconds with a higher percentage transmission at 1 minute and 5 minute infection feeding periods. No optimum infection feeding interval was found. Inoculation threshold was 5 minutes. In serial transfers single pea aphids could infect only 1 plant of a series. In colony transfers the virus persisted in the insect for 3 hours or longer. The virus was successfully transmitted from red clover, *Trifolium pratense*, alsike clover, *T. hybridum*, and yellow sweet clover, *Melilotus officinalis* to peas, *Pisum sativum* var. Perfected Wales. It is proposed that the Wisconsin pea streak virus be classified as a nonpersistent virus.

Electron microscopy of tobacco mosaic virus in vivo. SKOTLAND, C. B. Ultra-thin sections of healthy tobacco (*Nicotiana tabacum* L. var. Havana 38) tissue and tobacco tissue infected with tobacco mosaic virus were prepared for viewing in the electron microscope. Over 200 electron-micrographs were taken. These photographs indicated a relationship between the virus particles and dark granules, presumed to be grana. These dark granules were found in chloroplasts of both healthy and diseased tobacco tissue. In some cases the granules were found embedded in a colorless stroma; in other instances they were associated with fibrous masses. These granules were also found dispersed throughout masses of virus particle. In only 1 case were the virus particles found to be associated with chloroplasts as such. In this instance the rods formed a parallel layer around the periphery of 1 side of 2 chloroplasts.

The relation of Aceria tulipae Keifer to streak mosaic and other chlorotic symptoms on wheat. SLYKHUIS, JOHN T. *Aceria tulipae* Keifer infesting diseased wheat transmitted wheat streak mosaic and induced other chlorotic symptoms and a leaf roll on young wheat plants. Cultures of mites

developed from eggs transferred to disease-free wheat caused the characteristic leaf roll but no chlorotic symptoms until the mites became exteremely numerous. However, after these mites had fed on plants with symptoms of either streak mosaic or 2 types of a non-manually transmittable chlorosis, even small numbers of them induced the respective symptoms on disease-free wheat. All active stages of *A. tulipae* transmitted wheat streak mosaic. Although the virus persisted with the mites for several days, and was retained through the molting periods, there was no indication that it was carried through the eggs. Eggs and mites of all stages survived lower temperatures than Kharkov or Yogo wheat hardened 3 weeks at —5° C. Live mites and eggs were found on winter wheat collected at various times through the winter and spring. The mites were quickly eliminated when infested hosts were destroyed by cultivation. Wind dispersal of mites and hence of wheat streak mosaic has been demonstrated.

Infection of parsnip by Itersonilia sp. SOWELL, GROVER, JR. Germination of the ballistospores of *Itersonilia* sp., responsible for parsnip canker and leafspot in New York State, occurs in about 6 hours at 21° C. The germ tube may continue growth to form a mycelium or produce (1) an appressorium, (2) a binucleate conidium, or (3) a secondary ballistospore. Penetration of the leaf apparently is always direct and associated with the formation of an appressorium. In the epidermis the penetration hypha is inter-cellular. In the palisade layer it passes between the cells and down into the mesophyll layer. Here it branches and ramifies freely between the cells. Although details of actual penetration of the leaf have not been observed, it has been studied on colloidion membranes. Here penetration occurs by means of a peg produced on the lower surface of the appressorium about 8 hours after the spores are placed on the membrane. Only the dicaryotic form of the fungus has been isolated from nature. A monocaryotic form, produced in the laboratory, proved to be pathogenic on roots and leaves. Attempts to demonstrate the presence of mating types have failed.

The longevity of endoconidiophora fagacearum in lumber and other wood products cut from diseased trees. SPILKER, OREN W. Lumber and other wood products from diseased trees were placed under various conditions to determine whether *Endoconidiophora fagacearum* would survive and could be spread when these materials were utilized. Numerous isolations were made at the time of cutting and at approximately 3 week intervals. The fungus was never isolated from lumber or sawdust. It was isolated from slabs at the initial samplings; however, 3 weeks later the fungus could not be isolated from this material. *E. fagacearum* was isolated from branches, stumps, trunk, and roots when trees were cut. Samples taken 3 weeks later from branches and trunk did not contain the fungus. After 9 months, the fungus was still viable in the roots.

Strains of tomato bushy-stunt virus. STEERE, RUSSELL L. Through the use of a 3-host system 6 strains of tomato bushy-stunt virus have been isolated. Yellowish and whitish areas appearing in bushy-stunt infected plants of *Datura meteloides* 2–3 months after inoculation were ground and used as inoculum on *Nicotiana glutinosa* plants. Local lesions appearing thereon were ground and used as inoculum for plants of *Datura stramonium*. From numerous such passages 6-strains have been isolated which show essentially no differences when inoculated into *Datura meteloides* but which are distinctly different from each other in symptomology in *Datura stramonium* and show some differences in the symptoms which they produce in tomatoes.

Tobacco ringspot virus, a polyhedron, purified with the use of butanol. STEERE, RUSSELL L. Particles 25 mμ in diameter which have infectivity associated with them and which appear hexagonal in electron micrographs have been isolated from plants of "Caserta" squash infected with tobacco ringspot virus. These particles are concentrated and purified by use of a procedure in which the non-infectious plant components are removed from the juice of infected plants by emulsification of the juice with 1½ volumes of n-butyl alcohol followed by a series of low and high speed cycles in a "Spinco" preparative centrifuge. Healthy plants of the same squash grown simultaneously and passed through the same purification procedure yielded no such particles.

Infection of resistant varieties of wheat by races of Puccinia graminis tritici. SWAEBLY, MARY ANN, AND HELEN HART. Certain changes in host cells are common to all stem rust infections on resistant wheats, but development of some rust races or biotypes varies greatly on some varieties. Chloroplasts in host mesophyll corrode and disappear; cell walls change composition and thicken irregularly, some thickenings being discolored and wartlike; some cells die rapidly, others merely appear unhealthy. Race 15B does not readily infect Kentana, on which few uredia develop, but appressoria often form over stomata and develop no further. Nevertheless, host cells bordering the substomatal cavity sometimes appear injured by diffusible substances from rust appressoria. Biotypes of 15B develop differently on resistant Khapli emmer. In minute flecks produced by normal 15B, rust mycelium is restricted to substomatal areas and relatively few cells that soon die. In blotchy flecks produced by a Virginia biotype, mycelium is less restricted and runner hyphae grow out toward healthy host cells. An Oklahoma biotype produces profuse mycelium and some large uredia. Race 11, virulent on Kentana at 80–85° F., produces infection types O; and 1 at 70° F. and its mycelium is a restricted compact mass, its haustoria are small and shrivelled, its few runner hyphae branch abnormally, and its sporulation is sparse.

Possible means of overwintering of the horseradish white-rust fungus, Albugo candida. TAKESHITA, R. M., AND M. B. LINN. Since white-rust conidia cannot survive Illinois winters, other means of overwintering have been investigated. Oospores were first found in leaves of greenhouse-grown horseradish in 1951. They were observed in the field in September 1952, but only in limited quantities, being most abundant in the hypertrophied petioles of mature leaves, rare in roots and absent in leaves. Attempts to germinate oospores have been unsuccessful. Although systemic crown infections are fairly common, spread of the mycelium into the secondary roots (sets) which are used for next year's planting is rare. Mycelial spread downward is inversely related to the diameter of the root. White-rust affected roots can often be recognized by dark discoloration, hardness, and hypertrophy of the tissues. Such roots often succumb to various secondary rot-producing organisms. Localized infection of sets by zoospores from conidia may be another means of overwintering. Out of 1200 commercial sets examined in 1953, 18 roots were affected by white-rust and 8 of these showed localized infection. Sets may be exposed to infection during lifting or harvesting.

Fusarium stem rot of carnation. TAMMEN, JAMES. Using cross-pathogenicity tests, 75 individual clones of *Fusarium roseum* (Lk) Sny. and Hans. were studied to determine whether those clones reported to attack carnation were biologically distinct from the form *Fusarium roseum* f. *cerealis* or whether they were biologically identical to it. Results from these tests show that carnation clones of *Fusarium roseum* readily attack cereals and that cereal clones readily attack carnations. It is thus concluded that the clones of this fungus are not biologically specialized in respect to these 2 host plants and that the establishment of a new form within the species *Fusarium roseum* to distinguish the carnation pathogen does not appear to be justified.

Nematicidal potentialities of some agricultural pesticides based on contact, ovicidal, and therapeutic tests. TARJAN, A. C. Living specimens of *Panagrellus redivivus* immersed in 1000 and 100 p.p.m. suspensions or solutions of each chemical and observed at various intervals up to 24 hours indicated that Heptachlor 2E (1,4,5,6,7,8,8-heptachloro-3a, 4,7,7a-tetrahydor-4,7 methanoindene), Stauffer Experimental Compound N-244 (3-p-chlorophenyl-5-methyl rhodanine), and Hyman Experimental Compound 51-P-162 (hexachloro-

cyclopentadiene) were the most effective contact nematicides investigated. Ovicide evaluation consisted of immersing viable egg masses of *Meloidogyne incognita* in 1000 p.p.m. suspension or solutions of each chemical for 24 hours at 75° F. Treated egg masses were then placed around the roots of noninfected indicator plants. After 6–8 weeks' growth, root-knot indexes and relative numbers of resulting F_2-generation egg masses indicated that Stauffer N-244 and Heptachlor 2E were the most effective ovicidal chemicals investigated. Stauffer N-244, Heptachlor 2E, Hyman 51-P-162, Systox (0, 0-diethyl-0-ethyl mercapto ethyl thiophosphate), and Malathon (0,0-dimethyl dithiophosphate of diethyl mercapto succinate) had therapeutic properties when applied in water emulsion at the rate of 4 gms. or less per sq. ft. to soil in which *M. incognita*-infected tomato plants were growing. EPN-300 (ethyl p-nitrophenyl thionobenzene phosphonate), Vancide 51 (a mixture of the sodium salts of dimethyl dithiocarbamic acid and 2-mercapto benzothiazole), Chlordane (1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), Lindane (2-1,2,3,4,5,6 hexachlorocyclohexane), the sodium salt of 2-benzothiazolyl mercapto acetic acid, and Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5, 8a-hexahydro-1,4,5,8-dimethano napthalene) were found ineffective as nematicides.

Comparison of benzothiazolyl-2-thioglycollic acid derivatives with other chemicals for Dutch elm disease therapy. TARJAN, A. C., AND F. L. HOWARD. Field trials were conducted on about 200 nursery elms (1½–2½ in. d.b.h.), *Ulmus americana*, in which several laboratory-selected chemicals were applied singly either as protectants or eradicants to 5 randomized, replicate trees. Foliar sprays were applied on the same day that trees were inoculated with ±400,000 spores of *Ceratostomella ulmi*, and again 7 days later. Although the methyl ester and sodium salt of benzothiazolyl-2-thioglycollic acid, and "Nugreen" (44 per cent nitrogen) were effective in retarding symptoms temporarily, sprays containing Phygon (2:3-dichloro-1:4-naphthoquinone), Actidione (cyclohexamide), thiazole-2-thioglycollic acid, 4-methyl thiazolyl-2-thioglycollic acid, and 4 dyestuffs, Pontamine Blue, Chrysoidin RS, Helione A, and Erie Fast Orange, were ineffective. Solutions of Quenate (8-hydroxyquinoline benzoate), Trucidor (polynitrocycloaralcoyloxybenzene), salicylic acid, and Helione A injected into the soil 1 week before inoculation of trees with *C. ulmi* failed to prevent infection or to retard the disease when compared to inoculated, untreated elms. A spiral arrangement of holes bored in the trunk and filled with dry chemical yielded best results. Benzothiazolyl-2-thioglycollic acid, salicylic acid, Helione A, and 8-hydroxyquinoline benzoate applied prior to inoculation materially reduced symptoms, but were ineffective when applied following inoculation.

Control of tobacco blue mold by root application of Zineb and Ferbam. TAYLOR, GORDON S. AST shade tobacco plants sprayed with zineb (1½ lbs./100 gal.) and ferbam (2 lbs./100 gal.) received 3 washings, each equivalent to .18 in. of rain during 5 days before inoculation with *Peronospora tabacina*. On a scale of 0 = no disease, to 6 = severely diseased, zineb scored 1.3, ferbam 3.0, and check 4.3. LSMD 5 per cent was 1.4. This suggested a chemotherapeutic action for zineb following absorption from the wash water. To test this, zineb and ferbam (200 p.p.m.) were applied to roots of AST shade tobacco plants grown in sand. Without wetting foliage, 50 cc. of test solution were applied to each plant for 5 consecutive days. There were 3 treatment groups, ending 10, 6, and 1 day before inoculation with *P. tabacina*. The entire experiment was inoculated at 1 time. Plants were scored on the 0-6 scale after 2 weeks. Mean scores of 10 plants for zineb, ferbam, and water, respectively, were for 10 days 1.4, 2.2, 4.2; 6 days, 1.5, 0.8, 4.9; and 1 day, 1.0, 2.0, and 5.3. LSMD at 1 per cent was 1.3. Apparently both zineb and ferbam can act as chemotherapeutants under some conditions. This is in agreement with unpublished data kindly supplied by Dr. Saul Rich.

Virus-Like particles from some plants having symptoms of virus disease. THORNBERRY, H. H., A. E. VATTER, AND DONALD HICKMAN. Virus-like particles, revealed by electro microscopy, were obtained from leaves of some plants having symptoms of virus diseases by a purification procedure consisting of aqueous extraction, adsorption, elution, and differential ultra-centrifugation. Such particles were not observed in samples from healthy leaves subjected to similar treatments. Clarification sedimentations were at forces approximately 5000 × G. (gravity) for 20 minutes; virus-like particle sedimentations at 10,000 × G. or greater for 30 minutes or longer. Mounts for electron microscopy were shadowed with palladium. Materials processed and results obtained (approximate values) follow: elm phloem necrosis spheres 50 mμ; chokecherry x-disease spheres 50 mμ; cherry ring spot spheres 80 mμ; wheat mosaic rods 15 × 280 mμ (some spheres 40 mμ); sugar cane mosaic rods 20 × 150 mμ; oat red leaf rods 10 × 150 mμ or 10 mμ spheres in chains; orchid mottling rods 15 × 280 mμ; 13 viruliferous leafhoppers (*Colladonus geminatus*, Van D.) questionable spheres 50 mμ; 11 viruliferous leafhoppers (*Scaphytopius acutus*, Say) no recognizable particles; wheat rosette no recognizable particles. Infectivity of samples containing these particles has not been established.

Wilt-inducing protein-like pigment from Armillaria mellea (Vahl) Quel. isolated from peach roots. THORNBERRY, H. H., AND B. ROGER RAY. A dark brown protein-like pigment was obtained from a liquid, agitated culture of *Armillaria mellea* (Vahl) Quel. which was isolated from a root of a diseased peach tree showing wilting. The pigment was separated from the culture medium by adsorption to and elution from Celite. It was partially purified by differential sedimentation in an ultracentrifuge. The pigment at 0.32 per cent protein in 0.1 molar phosphate buffer solution at pH 8.0 had a sedimentation constant (Sw20) of approximately 2.5×10^{-13} sec., thus a molecular weight of approximately 30,000. It was not detectable in the electron microscope. At about pH 6.5 it aggregated into microscopically visible particles. At about pH 4.0 the aggregates were sufficiently large to settle by gravity. The substance was nontoxic to mice or *Bacillus subtilis*. It induced wilting in tomato seedlings and peach twigs at as little as 0.016 mg. protein per ml. and penetrated 15–20 mm. into the vascular tissues. The hydrogen-ion concentration of tomato stem and peach twig tissues is approximately pH 5.6. These data suggest that the fungal pigment aggregates at the pH of the plant sap and mechanically clogs the water-conducting tissues.

The survival of races of Phytophthora infestans in a mixture. THURSTON, H. DAVID, AND CARL J. EIDE. In 1952 it was reported that Cobbler potato plants were inoculated with a mixture of approximately equal numbers of sporangia of 2 isolates of *Phytophthora infestans* differentiated by their pathogenicity on Cobbler and Cherokee varieties. After 3 successive transfers to Cobbler, both Cobbler and Cherokee were inoculated, and Cobbler had 20 times as many lesions as Cherokee. During the past year a similar mixture of the two races was transferred on Cobbler for 9 successive generations. The proportion of the Cobbler isolate to the Cherokee isolate after each transfer was as follows: 3–1, 3–1, 2.4–1, 11–1, 12–1, 6–1, 14–1, 37–1, and 90–1. The Cobbler isolate alone has been transferred to Cobbler for 10 successive generations, and the Cherokee isolate alone has been transferred separately to Cobbler and to Cherokee for 6 generations. No apparent loss or gain of virulence was noted during these transfers. These results demonstrate a lack of survival ability of the Cherokee isolate in mixtures with the Cobbler or field isolate. They also indicate that races may be present in such minute quantities that they would not be noted until a selective variety was grown in considerable numbers.

Colletotrichum destructivum on brome grass. TIFFANY, LOIS H. Isolates of a straight-spored Colletotrichum were obtained from basal necrotic lesions on leaves of brome grass (*Bromus inermis*) collected in an alfalfa-brome grass pasture near Ames, Iowa, in August, 1952. Successful inocula-

tions were made on brome grass, red clover, sweet clover, and alfalfa in the greenhouse. The isolates from brome grass were identical in morphological and cultural characteristics with cultures of *Colletotrichum destructivum* from various legumes. In 1951, an isolate of the perithecial stage, *Glomerella glycines*, had been obtained from similar diseased material. Inoculations on brome grass and on various legumes were unsuccessful with the perithecial isolate. *Colletotrichum destructivum* has been found only in mixed brome grass-legume pastures, never in pure stands of brome grass.

Identification of seedling potatoes immune to virus X. TIMIAN, ROLAND G. An efficient means of identifying immune seedling plants in a potato progeny segregating for immunity to virus X has been developed. When inoculated with a highly virulent strain of potato virus X susceptible potato seedlings expressed necrotic local symptoms within 4 days. Optimum temperature for symptom expression of virus X in seedling potatoes was about 16° C. but symptoms were expressed over a range of 10°–24° C. when certain isolates were used. Effective mass inoculation of potato seedlings was accomplished by applying with a paint spray gun 12 gm. of 400 mesh carborundum in 100 ml. of inoculum. Ten isolates were compared on potato seedlings. A ringspot isolate, X 5, was best suited for mass inoculation because it incited both local and systemic symptoms on a higher percentage of plants than did the other isolates. Mechanical inoculation with a suitable isolate was as reliable as the conventional graft test in identifying immune clones in segregating progenies. All susceptible X-free potato plants tested expressed necrotic symptoms after being inoculated with a severe isolate of virus X. A seedling crop of potatoes virtually immune from virus X was obtained by mass inoculation of seedling plants before transplanting, followed by hand inoculation after transplanting to the greenhouse bench.

The inheritance of some characters in Helminthosporium sativum. TINLINE, R. D. Perithecia of *Cochliobolus sativus* (*Helminthosporium sativum*) have developed only when 2 compatible isolates are grown together on a suitable medium. White and black perithecia were produced when an albino strain and a "wild type" strain were grown in mixed culture. This constitutes evidence that isolates of the fungus are hermaphroditic. Ascospores, isolated at random from black or white perithecia, produced albino and "wild type" colonies in approximately equal numbers. The colonies were divided approximately evenly into the 2 mating types, regardless of color. When all 8 spores of an ascus were isolated individually, the factors for color and mating type were found to be inherited independently. Usually, the progeny segregated into 4 factor combinations in equal numbers. Occasionally, all the progeny were new combinations. Apparently color and mating type characters are controlled each by a single factor.

Cankers and decay of living yellow birch caused by Poria laevigata (Fr.) Cooke in the Monongahela National Forest. TRUE, R. P., E. H. TRYON, AND J. F. KING. Living yellow birches of large diameter having large bark-covered cankers as the only external indication of decay often show extensive heart rot. Eight large living yellow birches averaging 22 in. d.b.h. and having bark-covered cankers were felled and dissected. The decay found was a white rot composed of alternating concentric, or sometimes intersecting, broad or narrow zones of white and light reddish-brown wood. The rot was usually confined to the heartwood but sometimes involved the sapwood also. In the 8 trees examined, decay extended throughout the merchantable length. In 5 of these trees no external indication of decay other than the cankers was found. Dead branches of 2 trees bore sporophores of *P. laevigata* but fruiting bodies were not found associated with the cankers on living trees. At least 2 cankers showed the characteristic hard checkered surfaces described by Campbell and Davidson. Isolations made from these and similar cankered trees have consistently yielded *P. laevigata*. The height of the lowermost cankers ranged from 9–24 ft. and averaged 13 ft. above the ground. Trained foresters can diagnose the condition from the ground with reasonable accuracy even when trees show no other decay indication.

Effect of seed treatment on seedling stands and on hay yields of forage legumes and grasses. TYLER, L. J. R. P. MURPHY, AND H. A. MACDONALD. Seedling stands and yields of alfalfa, red clover, birdsfoot trefoil, smooth brome grass, orchard grass, Sudan grass, and timothy grown from seed treated with protective fungicides were compared with stands and yields of these species grown from untreated seed at Ithaca, New York. Seeds were sown broadcast without nurse crops in field plots on soils which varied in type, fertility, drainage and cropping histories. A randomized, split-plot planting plan was used. A single forage species formed the main plot which was split into 3 sub-plots to accommodate the treatments, viz. Arasan, 8 oz. per 100 lb. of seed, Phygon, 6 oz. and untreated seed. The area of each sub-plot was 100 sq. ft. Each treatment was replicated 6 times on each of 2 farms in each of 2 years (1950 and 1951) and on 1 farm in 1 year (1952). Plantings were made in late April or in early May; extensive stand counts were made 5 to 7 weeks later. Hay yields were obtained the second growing season for all species except for the annual Sudan grass. Statistical analyses of the data for each species showed that stands and yields from treated seed did not differ significantly from those produced by untreated seed.

Survival of chlamydospores, of Tilletia brevifaciens G. W. Fischer, exposed outdoors on soil. TYLER, L. J. Heads of winter wheat affected by the dwarf bunt fungus were collected in New York wheat fields in July, 1947. Sori were removed and dried in the laboratory and placed in small bags made of glass cloth tied with glass cord (¼ to ½ tsp. of sori per bag). August 20, 1947, 2 doz. bags of sori were placed outdoors on the soil on the experiment station farm. One-fourth-in. mesh rust resistant screen was appropriately placed to guard the exposed material. Germinability of exposed spores was tested at intervals of 6 months to 1 year. For each test a different bag of exposed sori was brought to the laboratory; spore material therein was placed in clean glass cloth and immersed in diluted commercial Chlorox (1 to 9) for 10 minutes. These surface treated spores were washed in water, placed on 2 per cent non-nutrient agar in Petri dishes and incubated at 6° C. Results obtained during the 5-year period show that every exposed sample of sori tested contained germinable spores. Spore germination was greatest (estimated 10 to 30 per cent) in samples exposed 6 months to 1 year and it diminished gradually with increase in the exposure interval. In December, 1952 a bare trace of germination was obtained in a sample of spores exposed continuously on the soil slightly more than 5 years.

Rapid spread of race 4 of Puccinia graminis avenae in Argentina. VALLEGA, JOSE, AND HUGO P. CENOZ. Races 7 and 3 of *Puccinia graminis avenae* were the only races found in Argentina from 1939 to 1949. During these years the variety Richland and its derivatives showed high resistance. In 1950 a few big pustules were seen on Richland hybrids and race 4 was determined by the senior author for the first time in this country. Only 2 years later (1952) race 4 was isolated from several places of Entre Rios and Buenos Aires Provinces showing a rapid and unexpected spread. This fact could be easily explained for 1 or 2 experimental fields where new selections resistant to races 7 and 3 are being increased but not for the oat growing area where farmers continue cultivating old varieties very susceptible to races 7 and 3 as well as to race 4. Also the 2 last years were not favorable for rust epidemics and crops severely infected were very scarce.

Microclimatic distribution of white pine blister rust in southwestern Wisconsin. VAN ARSDEL, E. P., A. J. RIKER, AND R. F. PATTON. Blister rust infection on eastern white pine was localized in certain places in southern Wisconsin while generally widespread in northern Wisconsin wherever ribes were present. To help explain this distribution, constant temperature studies of spore germination on agar

and of infection on host plants showed that aeciospores effectively germinated from 8 to 24° C. (optimum 16 to 20°); urediospores from 12 to 28° (optimum 20°). Teliospores produced on ribes at a constant 16° produced sporidia from 4 to 20°, teliospores produced at 20° were erratic in sporidial production, and teliospores produced at 24° C. were practically sterile. In "wave" years such as 1944 and 1947, the rust was intensified in the same areas rather than becoming generalized. Openings in the forest canopy, sheltered valleys, lower slopes, and kettle holes were such favored places. Hygrothermographs showed rust areas not only averaged less than 20° C. but also had a saturated atmosphere several hours more per day than the surrounding area in the late summer and fall. Certain topographic features and vegetation patterns that lowered temperatures and increased humidities were correlated with rust distribution in the areas studied.

Corticium praticola and the Rhizoctonia solani problem. VANTERPOOL, T. C. While working with *Rhizoctonia solani* strains isolated from blighted flax seedling it was found that the basidial stage of most of them could be obtained fairly readily in culture. Contrariwise, it has not been found possible to induce authentic strains of *R. solani* from potato to fruit. The flax strains produced sterigmata averaging 3 times as long (26μ) as those recorded for *Pellicularia filamentosa* (Pat.) Rogers (8–9μ). Further studies suggested that the flax strains correspond broadly to the type A strains of *R. solani* as delimited by Houston and the potato strains to his type C. Morphologically, culturally and pathogenically they belong to *Corticium praticola* Kotila. They also are readily isolated from alfalfa and sugar beets, and constitute the majority of the more pathogenic strains isolated from cereal seedlings. They show a slight aversion to the potato strains on culture plates, are faster growers, have a wider host range and are generally more pathogenic, and show growth inhibition to potato sprouts on plain agar plates. It is contended that *C. praticola* is a much more common pathogen than has been supposed and that many forms hitherto included in *R. solani* have *C. praticola* and not *P. filamentosa* as their perfect stage.

Dissociation and chromatic variation in Fusarium lini Bolley. VENKATA RAM, C. S. When a monoconidial culture of *Fusarium lini* grown on Richard's medium containing different carbon sources (olive oil, cellulose, lactose, sucrose, starch, glycerine, and urea) was transferred in mass inoculum to potato-sucrose agar, the original white isolate produced white, purple, and red colonies in the second cultural generation. Mass transfers from these 3 types gave numerous red and purple colonies that dissociated for color intensity in sectors. Monoconidial isolations from the various dissociants in the third cultural generation produced lines ranging in color intensity from very light to dark purple and red. All the 132 isolates comprised 11 distinct color groups, which retained their color distinction through 10 subsequent cultural generations on potato-sucrose agar. Preliminary results have indicated physiologic differences between the isolates belonging to these 11 color groups. In all, 132 culturally different isolates were obtained from a single spore during 13 cultural generations. In a similar experiment with various nitrogen compounds in Richard's medium, however, the original white isolate did not produce any colored lines.

Reduction in water flow by mycelium in tracheal elements. WAGGONER, PAUL E., AND A. E. DIMOND. The reduction of water flow by mycelium in Fusarium wilted tomato plants was estimated with a scale model where Reynold's number (R) is that encountered in the host. Tracheal fluid in healthy and diseased stems has the viscosity of water. It moves through healthy and diseased stems at 0.250 and .004 cm./sec. respectively. R is 0.01–0.25 in a healthy stem with vessel diameter (D) 5–100μ. Hyphae (diameter 3–5μ) are continuous through any perforation plates. The ratio of the drag coefficients (C) for different models was a constant at a given R. C in an obstructed vessel, relative to C in an unobstructed one, is 2 when plates with single perforations of 0.5D occur 12D apart. It is 4 when 2 hyphae of diameter 0.1D are also present. C is 1.7 when 2 hyphae alone are present. For 10 hyphae in a vessel without plates, C becomes 6. When a hypha of diameter 0.3D extends through a smaller vessel, C is 3. In an invaded vessel, the tension must increase 1.7– to 6–fold if flow is not decreased. Reduction in flow by mycelium must contribute to but cannot be the total cause of decreased water movement.—Atomic Energy Commission support is acknowledged.

The relation of host nutrition to the multiplication of tobacco mosaic virus in tobacco. WEATHERS, LEWIS G., AND GLENN S. POUND. The concentration of tobacco mosaic virus in tobacco growing under various levels of nitrogen, phosphorus, potassium, and concentration of balanced solutions was determined by local lesion assays to the F_1 hybrid of *N. tabacum* $\times$ *N. glutinosa* and by measuring the ultraviolet absorption (optical density) of purified virus preparations in a Beckman spectrophotometer set for a wave length of 265 mμ. The effects of increasing potassium and concentration of balanced solutions mainly reflected responses in host growth. With potassium the virus concentration curve was directly correlated with the growth curve of the host. With variations in the concentration of balanced nutrients there was a close correlation of host growth and virus concentration. The highest local lesion counts and optical density readings occurred at the levels of maximum growth. Increasing phosphorus resulted in corresponding increases in virus concentration even though at excess phosphorus levels growth was markedly stunted. The infectivity of crude expressed juice from plants receiving variations of nitrogen was inversely correlated with the increase of nitrogen. The virus concentration of purified virus preparations, however, was directly correlated with increase of nitrogen. This discrepany was attributed to an inhibitor in crude extracts which increased with increase in nitrogen. In purification the virus was freed from the inhibitor.

The pycniospore stage of Cronartium quercuum and C. strobilinum. WEBER, GEORGE F. Droplets of syrupy exudate containing pycniospores were observed in the early morning on woody galls of *Cronartium quercuum* on *Pinus taeda* in the Gainesville, Florida area on November 15, 1952. They have not been observed previously in nature on the host in the state. Some pycnia appeared to have matured 10–14 days earlier and they continued to develop until early December. They were subcuticular, confluent, imbedded in the inner cortex with no conspicuous openings, and occupied a thin layer of host tissue. Often the bright color of the spores showed through the outer cortex. Pycniospores were obovoid, almost teardrop shape, 3.2 $\times$ 4.3μ, wall hyaline and contents orange colored. The aecia developed February 15 continuing for about a month. Uredinia and telia were evident after March 15 on the oaks completing a 2 year cycle. On *Pinus caribaea, Cronartium strobilinum* pycniospores appeared March 15, 1953 on young cones that developed on the spring growth. The aecia on the cones were shedding mature spores 2 weeks later. Uredinia and telia developed on the oaks later in the season insuring inoculum for cone infection after December thus completing a 1 year cycle.

The etiology of false smut of buffalo grass (Buchloe dactyloides) caused by Cercospora seminalis E. and E. WEIHING, JOHN L. Hyphae of *Cercospora seminalis* penetrate the stigma of an unfertilized ovary of buffalo grass, grow down the style and into the ovary. The mycelium generally follows the cell walls. In the ovary the initial invading strands of mycelium traverse to the funicular tissue but do not parasitize it immediately. The mycelium then grows throughout the remaining ovary tissues, onto the ovary surface and into the style. The entire ovary cavity, delimited by the lemma and palea, is eventually filled by the fungus. The mycelium emerges from the summit of the lemma and palea, grows equally in all directions and forms spores periodically at the hyphal tips. Hyphae arise below the scar tissues formed by spore abscission thereby permitting continuous growth. The unrestricted mycelial

growth outside the enclosing floral structures causes the formation of a black bulbous structure 2-3 mm. in diameter atop each infected ovary. Young mycelium is thin-walled and colorless but with age it becomes thick-walled, highly septate and dark brown to black in color. No floral parts other than the ovary tissues become parasitized. Mycelium of *C. seminalis* can overwinter in old infected flower burrs or in the soil. Infection is completely inhibited by fertilization of the ovary.

Linkage groups in Glomerella cingulata. WHEELER, H. E. Morphological and biochemical mutants of *Glomerella cingulata*, most of which were radiation induced, were used in an attempt to establish linkage groups in this fungus. The inheritance of 40 mutant characters was studied by isolating and culturing the spores from each of more than 1500 asci produced by 60 different crosses. Each of these mutant characters appeared to be controlled by a single gene. Analysis of segregation data indicated 4 linkage groups representing at least 3 chromosomes: group I with 5 points, group II with 4 points, and groups III and IV with 2 points each. Group I included the A and B loci at which mating reactions are controlled. Three alleles at each of these loci have been identified previously and 1 of the mutant genes studied apparently represented a fourth allele at the B locus. In addition to providing stocks with as many as 6 genetic markers, the findings demonstrated that the wild-type and mutant cultures used in previous studies were haploid in nature.

Reaction of Cotton Varieties to Verticillium Wilt. WILES, A. B. In certain years Verticillium wilt is a destructive disease of cotton in the lower Mississippi Valley and in the irrigated areas of the Southwest. Using a seedling inoculation technique, greenhouse studies were made on the reaction of numerous varieties and selections of *Gossypium hirsutum* to *Verticillium albo-atrum*. The reactions of several varieties of *G. barbadense* and *G. arboreum* were also studied. Plants grown in seed flats were inoculated in the 4-leaf stage by removing them from the soil, dipping the roots in a suspension of the organism, and re-setting. Final readings were made about 4 weeks after inoculation using living (resistant) and dead (susceptible) classifications. Varieties of *G. barbadense* and *G. arboreum* were more resistant to Verticillium wilt than varieties of *G. hirsutum*. The highest degree of resistance in *G. hirsutum*, as measured by surviving plants, was found in the varieties Smith 81-14, Auburn 56, Alabama Hybrid 257-202, and Hartsville, in the order listed. From these tests individual surviving plants of the above varieties were grown to maturity and selfed. When progenies of these plants were tested a more uniform resistance was obtained than from the parent variety.

Berberis Thunbergii, a host of cucumber mosaic virus (Marmor cucumeris). WILKINSON, R. E. Japanese barberry bushes in New York State frequently have mosaic characterized by reddish blotches on the leaves. Varying degrees of distortion and reduction in size of leaves occur on different plants and even on a single plant. The virus is easily transmitted mechanically to tobacco from succulent barberry tissue, such as develops in spring or after the bushes are pruned in summer. Six isolates of the virus which were studied produce etch-type ringspots on the inoculated leaves of tobacco. Systemic infection in tobacco produces mild mottle with practically no distortion of the leaves. These isolates show promise for use as the challenge strain in cross protection tests. Half leaves of tobacco inoculated with known strains of cucumber mosaic virus did not produce ringspots when rubbed 4 days later with isolates from barberry. However, tobacco plants infected by 2 isolates from barberry were not completely protected against systemic invasion by a known strain of cucumber mosaic virus, although symptom expression was delayed and reduced. Symptoms characteristic of those which occur in nature were reproduced by mechanical inoculation of barberry seedlings with a known strain of cucumber mosaic virus. The virus was recovered from these mechanically infected bushes.

Effect of pH of Colletotrichum lagenarium conidial suspensions on number of lesions developing on cucumber leaves. WILLIAMS, LANSING E. The optimum initial pH for germination of conidia of an isolate of *Colletotrichum lagenarium* in 2 per cent glucose has been reported as 3.5, with a second optimum at 11.5. Using the same isolate, conidial suspensions of various buffered and unbuffered pH values were used to inoculate seedling cucumber leaves. Most lesions developed using suspensions at pH 5.5–6.5 and at pH 7.5. At pH 7.0, the number of lesions per leaf averaged 50 per cent less than at the maxima. The difference between the number of lesions at pH 7.0 and the maxima was greater on young leaves and less on older leaves. Using unbuffered conidial suspensions on the same series of plants, the maximum number of lesions sometimes developed at slightly different pH levels on young leaves than on old leaves.

Methyl bromide injury to some ornamental plants. WILLIAMSON, C. E. In April 1950 carnations planted in methyl bromide fumigated soil were injured severely. Injury consisted of permanent wilting of leaves, shoots, or entire plants. Affected portions died and became straw color. Rooted chrysanthemum cuttings wilted and died when planted in soil that received an excessive amount of methyl bromide. Fumigation experiments conducted at several locations produced interesting results. At one location all carnations planted in treated soil died, at a second about half of the plants survived, and at a third all plants survived although some injury occurred. Analyses of soil samples taken before and after fumigation failed to show any significant change in nitrogen, potassium, phosphorus, calcium, manganese, ammonia, pH, or total salts. Mechanical analysis of the soil at the 3 locations showed, respectively, 9.4 per cent, 6.4 per cent, and 2.4 per cent colloidal clay. Further experiments demonstrated that injury is proportional to the amount of colloidal clay and organic matter present. Aeration, heat, pH change, or addition of calcium salts failed to reduce or eliminate injury. Heavy, repeated leaching with water was slightly beneficial. Sodium bromide mixed with soil produced injury symptoms on carnation similar to those caused by methyl bromide. Bromine released by decomposition of adsorbed methyl bromide may be the cause of injury.

Polyelectrolytes as spray conditioners. WILSON, J. D., AND J. B. MILLER. The adhesion of a fixed copper (tribasic) to tomato leaves was very substantially increased by the use of various polyelectrolytes, as was the period over which the fungicide remained suspended in water after thorough mixing. Alkaline polymers with high viscosity and high molecular weight gave better adhesion than those of neutral or acid reaction; acid polymers were improved in their adhesive action by neutralizing them with sodium carbonate or ammonium hydroxide. Alkaline polymers also gave better suspension than those of acid reaction. Interestingly enough, a standard soil conditioner formulation which can be prepared as a 325-mesh powder and mixed and stored with fixed coppers gave as good results as any of the polymers tested. The adhesive and suspending action of these materials on organic fungicides will be investigated during the summer of 1953.

Metabolites of vascular Fusaria of cabbage and cotton in relation to resistance. WINSTEAD, N. N., AND J. C. WALKER. Heated dialyzed replacement culture filtrates of the cabbage yellows and cotton wilt fungi were poured daily over the roots of susceptible and resistant plants of cabbage and cotton, respectively, growing in quartz sand watered with nutrient solution. Susceptible cabbage plants having 4 or more expanded leaves when treatment began showed veinclearing and yellowing of cotyledons and lower leaves after 48 hours, suggesting the earliest symptoms of yellows. Resistant plants with similar treatment remained free from these effects. Susceptible cotton plants within 6 days showed external symptoms similar to those of infected cotton plants, while resistant plants remained free from symptoms. No vascular browning was noted. The effects were specific for the susceptible variety of the host sus-

cept. Similarly prepared filtrates from other vascular fusaria had no effect. It is suggested that the cabbage and cotton forms of *Fusarium oxysporum* produce thermostabile, dialyzable metabolites which are specific for susceptible plants of the suscept and may in some way aid in the establishment of infection by the pathogen.

Production of vascular browning by metabolites from several pathogens. WINSTEAD, N. N., AND J. C. WALKER. The *Fusarium* wilt organisms affecting tomato, cotton, cabbage, and peas were grown in wheat bran cultures. The filtered washings were assayed for pectinesterase and polygalacturonase and each filtrate was introduced into cut stems of resistant and susceptible varieties of all 4 species of plants. All showed high pectinesterase and low polygalacturonase activity and each induced vascular browning in all test plants as shown for tomato by Scheffer and Walker. Similar results were secured with *Pseudomonas solanacearum*, and 3 root-rot fusaria, and for all organisms when replacement cultures on liquid media were used. No browning was secured when filtrates were heated, and no pectinesterase activity was found in such filtrates. Replacement culture filtrates of *Botrytis cinerea* were low in enzyme activity and produced no browning; bran culture filtrates were high in both enzymes and induced browning and soft rot of the lower stem. The browning factor was common to all of the organisms and was not specific for the respective susceptible host. The establishment of the pathogen in its respective susceptible host plant is therefore due to other factors than pectinesterase activity but after establishment in the xylem the latter enzyme is important in initiating vascular browning.

Relative sensitivity of single conidial isolates of Monilinia fructicola and Fusarium oxysporum lycopersici to different concentrations of certain fungicides. WOLLMAN, E. S. H. The possibility of increase of field races of pathogens that are resistant to specific fungicides is an important consideration in control of plant diseases by chemical means. Likewise, in the screening and evaluation of new chemicals as fungicides, differences in environment and the genetic variability of test organisms may contribute to conflicting data obtained in different laboratories. Single conidial isolates of *Monilinia fructicola* and of *Fusarium oxysporum lycopersici* were tested against commercial fungicides at different concentrations using the thread-plate method. Differences in sensitivity between isolates were observed, and these differences were consistent within replicates of a single experiment. Using the same isolates and concentrations of fungicides in other experiments did not always result in similar data. The effect of temperature previous to and following treatment of the infested threads was studied.

Acquired resistance to tobacco mosaic virus in bean. YARWOOD, C. E. The permanent localization of tobacco mosaic virus (TMV) lesions surrounded by an abundance of uninvaded and previously susceptible bean leaf tissue suggests a type of resistance induced by infection. The corresponding cessation within 3 days after inoculation of the initial rapid increase in virus supports this idea. When TMV was inoculated on bean leaves infected 2 days earlier with the same virus, lesions from the second inoculation did not develop close to the lesions from the previous inoculation. When non-invaded tissue adjacent to infected tissue was macerated and added to TMV inoculum, the infectivity was usually reduced more than by a similar addition of tissue distant from the infection. When non-invaded tissue adjacent to infected tissue was macerated, centrifuged, and the supernatent added to purified TMV suspension and heated, a heavier precipitate usually resulted than from a similar addition of extract from tissue distant from the infection. These results could be interpreted to indicate a type of acquired immunity due to antibody formation.

A mycorrhiza-like fungus in potato tissue cultures. YOUNG, DONALD A., ALBERT C. HILDEBRANDT, G. H. RIEMAN, AND D. C. COOPER. In examinations of over 400 tubers from over 10 varieties and 30 unnamed seedlings of *Solanum tuberosum*, the presence of a mycorrhiza-like fungus was confirmed. Further studies showed a similar fungus in tissue cultures derived from tubers of the varieties Katahdin, Kennebec, and Russet Burbank grown 18 months *in vitro*. The fungus also was found in recently isolated strains of tissue derived from the pith regions of Hindenburg, Sebago, and Russet Burbank tubers, and the pith and vascular regions of Katahdin tubers. Cultures derived from stems of Irish Cobbler, from an unnamed seedling, and from leaf petioles of Irish Cobbler similarly showed the fungus. Potato callus cultures all grew well on a simple agar medium containing mineral salts, sucrose, coconut milk, and 2,4-dichlorophenoxy acetic acid. The fungus was detected microscopically in freehand aceto-carmine stained sections from tissue cultures and from fresh tuber tissue. The fungus failed to grow either on the tissue culture medium or on an agar medium commonly used to reveal contaminants. No spores were found in tuber or callus cultures.

Resistance to Phytophthora root rot of avocado. ZENTMYER, GEORGE A. The root rot caused by *Phytophthora cinnamomi* is a serious problem in avocado culture in California. The search for a resistant rootstock recently has emphasized testing of avocados or related species of *Persea* which in their native habitat grow in wet, poorly drained sites; an ideal situation for resistance would also include presence of *P. cinnamomi* in such sites. In 1952 collections were made from 6 species of *Persea* growing in wet locations in Central America and Mexico: *P. americana, P. Donnell-Smithii, P. gigantea, P. Popenoei, P. schiedeana,* and *P. Skutchii*. In Mexico *Phytophthora cinnamomi* was found on roots of healthy *Persea americana* and *P. schiedeana* in wet sites. Tests in infested soil show that *P. Skutchii* seedlings have some resistance; the other species are being propagated for testing. Seedlings of *Persea borbonia* (provided by C. A. Schroeder) from swampy areas in the southern United States are either immune or highly resistant, but the species is apparently incompatible with avocado. Several seedlings from named avocado varieties growing in southern California have withstood infection in tests for Phytophthora resistance in beds of infested soil, and the clones are being propagated vegetatively for further testing.

C^{14} as a tool for the study of the oak wilt fungus. ZUCKERMAN, BERT M., AND P. F. HOFFMAN. *Endoconidiophora fagacearum* was grown on a C^{14}-labeled medium in a study of certain morphological and physiological characteristics. A sucrose-asparagine-yeast extract medium in which C^{14}-labeled sucrose was incorporated at levels of 10, 20 and 30 microcuries per ml. was used. The fungus grew normally upon transfer to unlabeled medium after having been incubated for periods up to 56 days on the labeled medium. Comparison showed no morphological differences between labeled and unlabeled conidia and mycelia of the same isolate. No differences were noted in the rates or percentages of germination of labeled and unlabeled spores. A labeled strain crossed with an unlabeled strain of opposite compatibility type produced fertile perithecia. The labeled fungus retained the ability to produce a metabolite toxic to tomato and oak cuttings. Paper chromatograms of filtrates from labeled and unlabeled cultures gave similar RF values. Labeled conidia and ascospores were pathogenic to red and live oak seedlings. Activity could be detected in leaves and at cut ends of stems with a G-M monitor. Autoradiographs of germinated spores, mycelia, and wilting leaves were obtained on Eastman No-Screen X-Ray emulsion and of germinated spores on several Ilford nuclear track emulsions.

STUDIES ON FUSARIUM WILT OF FLAX [1]

Byron R. Houston and P. F. Knowles [2]

The Fusarium wilt disease of flax caused by *Fusarium oxysporum* f. *lini* (Bolley) Snyder and Hansen has gradually spread throughout the flax growing areas of Imperial Valley and San Mateo County in California since the beginning of commercial flax production in 1934. The spread has been relatively slow if one considers that the varieties Punjab (C.I. 20), Punjab 47 (C.I. 1115), Imperial (C.I. 1114) and Argentine (C.I. 463), comprising most of the acreage, are extremely susceptible varieties. In the Imperial valley the first report of Fusarium wilt was in 1945 (9). Since that time the disease has generally spread over the area; however, losses have been quite low with a few exceptions. The causal organism does not seem to be readily adapted to the area as a soil inhabitant since planting nonsusceptible crops for a relatively short period of 3–4 years appears to reduce greatly the degree of infestation. Exceptions have occurred when rotations included irrigated summer crops such as alfalfa. Continual cropping to flax, however, does result in increased losses. In the other major flax growing area, San Mateo county, the disease was first reported in 1949 by Houston and Knowles (10). The spread in this area has been rapid and in some cases losses have been severe. This more nearly parallels the reports from other parts of the United States by Bolley (3) and Bolley and Manns (4).

Studies were undertaken to determine reasons for the variation in severity of flax wilt disease in the 2 principal flax growing areas of the state, as well as the influence of soil type and temperature upon disease development; also, to devise a rapid and uniform method of artificial inoculation so as to facilitate development of adapted resistant varieties and the study of inheritance of resistance.

METHODS OF TESTING AND PROBLEMS INVOLVED.—Since the work of Bolley in 1901 (3) explaining the cause of flax wilt, a great deal of work has been done in selecting and breeding flax for resistance to the disease (3, 13, 18). Testing of selections and crosses has been done in naturally infested field plots that have been continually planted to flax for many years. In a few cases both greenhouse tests and field plots have been used in selection as done by Millikan (13). Certain problems arise when using field plots in testing for resistance and studying the mode of inheritance. Bolley (3), Kommedahl (12), and others have shown that there may be great fluctuations from year to year in the relative resistance of varieties. Part of this is undoubtedly due to differences in environment from year to year as shown by Tisdale (19) and Kommedahl (12). It has also been shown by Borlaug (5), Broadfoot and Stakman (7), Millikan (13, 14), Tochinai and Takee (20) and others that physiologic specialization occurs in the causal fungus, consequently fluctuation of the strain predominating in any given plot may occur from year to year. Such a fluctuation has been observed in a naturally infested field plot in San Mateo county in which over a 3 year period there has been a change in the predominating pathogenic race. In this case the change appears to have been brought about by planting a large portion of the plot to varieties and selections resistant to the original pathogenic race and susceptible to the one now predominating. Millikan (13) has shown that infested soils from different areas may contain different physiologic strains of the fungus based upon pathogenicity. In California, plantings made in naturally infested soil nearly always show some plants being killed by *Rhizoctonia solani* Kühn. Losses have been extremely heavy in such soil planted to flax in the greenhouse, thus rendering the tests of little value in determining the wilt reaction. It became evident early in this study that the use of field plots or naturally infested soil in the greenhouse would not suffice in making an accurate determination of the resistance of varieties and progenies and the effect of environment upon disease development.

Greenhouse methods of inoculation.—The use of mass infestation of soil with agar or oat cultures of the organism made it very difficult to regulate the inoculum potential at the same level in each trial. Also preliminary trials showed that many plants of nearly any variety, regardless of its previously recorded degree of resistance, could be killed by pre-emergence or seedling infection if a heavy application of inoculum was used and environmental conditions were optimum for the fungus.

The method of inoculation found to give the most consistently uniform results involved the use of steam sterilized soil well adapted to the organism to which is added a spore suspension at the time of planting (11). The first wilting symptoms were visible 7 to 10 days after planting. Counts were made and wilted plants removed at about 4-day intervals during the first 2 weeks and less frequently thereafter. All the plants of susceptible varieties usually wilted between 25 and 35 days after planting. Greenhouse temperatures were maintained at about 25° C. Precations were taken to prevent infestation of the soil with any other fungus, such as *R. solani*, which might caused seedling damping-off. Occasionally the larvae of the mushroom gnat (*Sciara sp.*)[3] have attacked the seedlings by penetrating the hypocotyl just below the soil line. Such attacks result in symptoms similar to early stages of wilt.

[1] Accepted for publication April 5, 1953.

[2] Associate Plant Pathologist and Assistant Agronomist, respectively, University of California, Davis, California.

[3] Identified by Dr. Arthur E. Pritchard, Department of Entomology, University of California, Berkeley, California.

Control appears to be possible by using suitable fumigation to eliminate the adult and by applying a spray of chlordane (octachloromethanotetrahydroindane) to the soil surface once or twice during the course of the trial.

This method of greenhouse testing has been used successfully in conjunction with field plots to develop 2 wilt resistant varieties. A Punjab (C.I. 20) selection was released as wilt resistant in 1953 and a highly resistant Dakota (C.I. 1071) selection was released in 1952. The test is also being used in a study of the mode of inheritance of wilt resistance.

Selection and maintenance of pathogenic clones of the organism.—A number of publications on Fusarium wilt of flax deal with the physiologic and pathogenic differences existing between isolates of the causal fungus. In the past, isolates or groups of isolates have been referred to as strains (13), physiologic forms (5, 6, 17), physiologic races (4, 10) and pathogenic races (5). Such subdivisions have been made largely upon pathogenicity or on a combination of morphology, nutrition and pathogenicity (17). Snyder and Hansen (16) placed all the former species in the wilt Fusaria under the common species *F. oxysporum* with the subspecies designated *formae* as in *F. oxysporum* f. *lini*. Armstrong and Armstrong (1) have described biological races 1 and 2 of *F. oxysporum* f. *tracheiphilum* on cowpea as subdivisions of this *forma*. It seems clear that a descriptive term should be used to designate the subgroups under the *formae* of the wilt Fusaria. Neither the term physiological race nor biological race are sufficiently descriptive to be used when the only physiological reaction upon which the race is based is pathogenicity. It is felt that *pathogenic race* (5) should be used to designate those subdivisions of the *formae* based upon relative pathogenicity of the isolates on a number of varieties of flax. The term *clone* (17) can be used to designate the individual isolate which is maintained by single-spore transfers and selection.

Natural clones have been obtained from about 35 infested areas in various fields in the 2 flax growing regions. All clones obtained from aerial portions of the plants were of a type producing considerable coarse aerial mycelium with an abundance of microconidia and few macroconidia produced in sporodochia. Variations between clones were noted with respect to mycelial and substrate color, growth rate, and abundance of conidia. Single-spore transfers from the original clone were usually quite consistently the same as the primary type; however, subsequent single-spore transfers from original single-spore cultures that had been held in culture tubes for several weeks nearly always yielded 2 types of cultures, thus exhibiting the Hansen (8) dual phenomenon. The common secondary type mutant appeared to be the same as the type II described by Oswald (15), commonly referred to as the pionnotal type, with appressed growth and a heavy production of macroconidia. Most isolates upon repeated mass transfer change to this type. After such a change occurs single-spore colonies from these cultures have shown no reversion to the original parental type. All pionnotal types tested have had a lower virulence than the original isolates. Mutants other than the pionnotal type have been obtained by single spore transfer and all have shown lower virulence than the primary type. Maintenance of the natural clonal lines is accomplished by always using the single-spore technique in making transfers and selecting the natural type each time. Frequent re-isolation from inoculated plants also aids in preserving the natural type. Dried stems of diseased flax can be held for several months and the same natural type can be obtained when needed.

Selection of clones for use in greenhouse tests was based on their degree of pathogenicity to a range of varieties. The clones with the highest virulence which were pathogenic to the greatest number of varieties were used.

EFFECTS OF STEAMED SOIL AND TEMPERATURE UPON GREENHOUSE TESTS.—*Temperature.*—It has been clearly shown by Borlaug (5), Tisdale (19) and Millikan (13) that temperature may have a profound effect upon wilt development in flax. In greenhouse tests it is important to maintain conditions as near optimum as possible for wilt development. A greenhouse test in which 60 single plant progenies of Dakota (C.I. 1071) were inoculated and kept at an air temperature of 18 ± 2°C. showed an average wilt of 25.1 per cent after 45 days, whereas with the same progenies growing at the same time at a temperature of 24 ± 2°C. 50.5 per cent of the plants showed wilt, or approximately twice that occurring at the lower temperature.

Soil type.—The soil type can have a marked affect upon development of wilt. Repeated greenhouse plantings in naturally infested soil from Imperial Valley showed only 10-50 per cent of the wilt developing in comparable plantings in soil from San Mateo county. Also, it has been impossible to establish a uniformly infested plot in the field in Imperial Valley with repeated artificial infestation. The relation of soil type to disease development was determined in the greenhouse by using steamed soils from 3 different locations in California. Soil obtained from Imperial Valley was of sandy-clay-loam type with an extremely low organic content and a pH of 8.1; the Davis soil was of the Yolo sandy-loam series of pH 7.4 and contained a moderate amount of organic matter; the coastal soil (San Mateo county) was a sandy-loam with extremely high organic content and a pH of 5.6. The pH of each soil as given was taken after steam sterilization. Inoculations were made in each steamed soil type with the technique previously described (11). Conclusions on the effect of steamed soil type were based upon the relative pathogenicity of two Fusarium clones (No. 286 and 287) obtained from wilted plants in Imperial Valley when used to inoculate 4 varieties of flax. Six replications of 50 seeds of each variety were inoculated with each clone. The resulting percentages of disease 6 weeks after planting are given in Table 1. Both isolates killed 100 per cent of Punjab 47 in each soil type. In the Imperial Valley soil the varieties De Anza,

TABLE 1.—*The influence of soil type upon the percentage of infection by 2 clones of F. oxysporum f. lini when tested on 4 flax varieties in steam sterilized soil*

Flax variety	C.I. No.	Imperial Valley Soil Clone 286	Imperial Valley Soil Clone 287	Davis Soil Clone 286	Davis Soil Clone 287	Coastal Soil Clone 286	Coastal Soil Clone 287
Punjab 47	1115	100[a]	100	100	100	100	100
DeAnza	1156	2	9	8	56	7	52
Signal	1157	1	4	3	40	17	56
U.Sask. #2219		0	11	6	33	18	93

[a] Per cent of plants killed 6 weeks after planting.

Signal, and U. of Sask. 2219 showed a high degree of resistance to both clones, slightly more to 286 than 287. In the Davis soil, however, clone 287 was much more pathogenic on these same varieties. In the coastal soil isolate 286 was somewhat pathogenic and the virulence of clone 287 on U. of Sask. 2219 was greatly increased with a disease percentage of 93 as compared to 33 in the Davis soil. These results may help to explain why varietal reactions to wilt in one area of the country might be markedly different from that in another area whether tests were conducted in field plots or in the greenhouse. Furthermore, in testing relative pathogenicity of clones one would have to conclude that the 2 above clones were identical if tests were made in the Imperial Valley soil, whereas the 2 would be classed as markedly different when tested in Davis or coastal soil. Clone 287 could be classed in 3 different pathogenic races on the basis of its relative pathogenicity on the 4 varieties in the 3 soil types. Thus, it is of utmost importance in such tests that the soil type be well adapted to the fungus and that the same type should always be used. In all subsequent tests the coastal soil was used.

The exact nature of the effect of soil type on the wilt organism has not been determined, but the greatest differences between the soils was not in their physical structure but in organic content and pH. The growth of flax was very similar in steamed soil of the 3 types.

PATHOGENIC RACES OF THE FUNGUS.—Many workers have studied isolates of *F. oxysporum* f. *lini* and concluded that on the basis of pathogenicity races do exist (2, 5, 7, 14, 20). In this study the pathogenicity of 18 clones has been tested on 6 varieties of flax using the methods described (11) and using 6 randomized replications for each clone. The clones were selected at random from many which had been obtained from various areas, including Arizona, as well as 2 obtained from Canada through the courtesy of Dr. W. E. Sackston of the University of Manitoba. In general the clones could be grouped in 5 pathogenic races based upon the percentage of disease in each variety, using 20 per cent as a sufficient difference to warrant a class separation (Table 2). Pathogenicity was not correlated with the source of the clone as shown in 1 group where 3 clones of the same pathogenic race had their origin in different areas. The 1 clone from Canada differed from all others in that it was low in virulence. Low virulence is not uncommon in clones and is particularly true of many mutants arising in culture; however, low virulence probably should be considered as indicative of a pathogenic race.

The percentage of disease reported in this trial was calculated at the end of 45 days, so one cannot assume that all the clones would have fallen in the same race group as shown after a 60 or 90 day period. An example of this time relationship is shown in Figure 1 where the percentage of wilt in the varieties Royal (C.I. 828) and DeAnza (C.I. 1156) is plotted over a 30 day interval. The 2 clones of the fungus, 287 and 294, which in Table 2 are grouped in the same pathogenic race after a 45-day test, would not fall in the same group on the basis of their relative pathogenicity at 14 or 23 days after planting the seed. Clone 287 had killed 92 per cent of the Royal plants at the end of the 14-day period, but only 18 per cent of the De Anza plants. Clone 294, on the other hand, attacks De Anza more vigorously than it does Royal in the early stages of the test (9 days) and at 14 days had caused nearly the same percentage of disease in both varieties, but with only 42 per cent wilt in Royal as compared to 92 per cent on the same date with clone 287. With the relative pathogenicity of the 2 clones on

TABLE 2.—*Variations in pathogencity as shown by per cent of plants killed 45 days after planting by 10 clones of Fusarium oxysporum f. lini when tested on 6 flax varieties. The clones are grouped into 5 pathogenic races*

Clone No.	Source of Clone	Punjab 47 (CI 1115)	Royal (CI 828)	DeAnza (CI 1156)	Dakota (CI 1071)	Crystal (CI 982)	Redwood (CI 1130)
287	Imperial Valley	100[a]	100	*50*[b]	20	10	10
294	San Mateo Co.	90	90	*60*	30	10	10
310	San Mateo Co.	100	100	10	20	*40*	20
313	San Mateo Co.	100	90	10	20	*50*	20
307	Yuma, Ariz.	100	90	20	20	10	*0*
308	Imperial Valley	90	90	10	10	10	*0*
310-2	San Mateo Co.	100	90	10	10	10	*0*
311	San Mateo Co.	100	*70*	0	*0*	20	10
312	San Mateo Co.	90	*50*	10	*0*	30	10
355	Canada	20	20	0	0	0	0

[a] Percentage of disease to the nearest 10 per cent.
[b] Most important racial differences italicized.

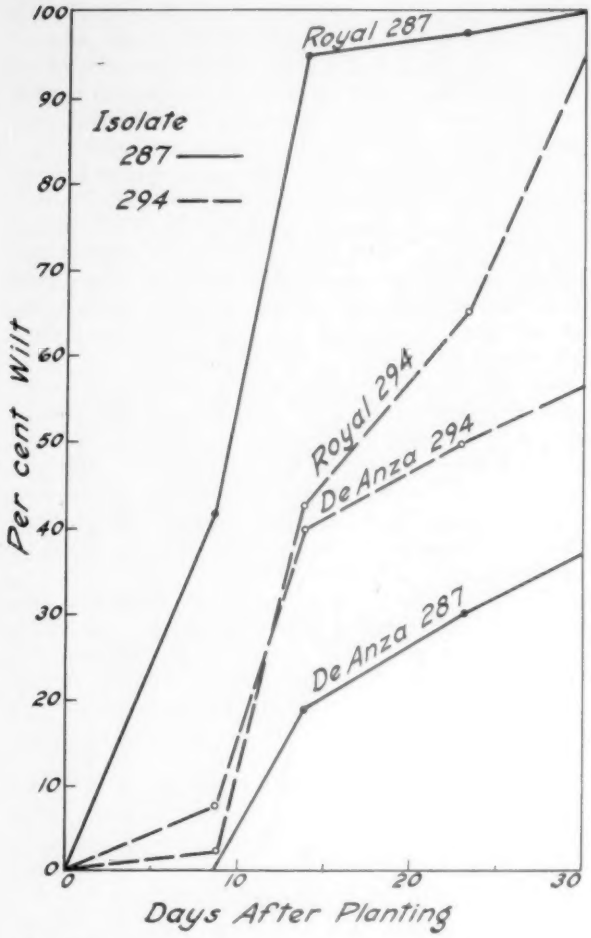

FIG. 1.—Relative pathogenicity of 2 clones of *F. oxysporum* f. *lini* on the 2 varieties of flax, Royal and DeAnza. Percentage of wilt plotted over a 30-day period. Note the reversal of relative pathogenicity of the 2 clones on the 2 varieties. At the end of 45 days these 2 clones showed approximately the same degree of pathogenicity on the varieties shown (Table 2).

the 2 varieties thus reversed in order at an early stage of the test this difference cannot be due to a difference in virulence or inoculum potential and must be considered as a difference in relative pathogenicity. This same relationship was also noted on other varieties and with other clones.

VARIATIONS IN SINGLE PLANT PROGENIES.—The greatest difficulty in attempts to determine true races of the flax wilt Fusarium lies in the fact that most of 10 flax varieties tested do not show a homogeneity of genotypes in regard to resistance to the disease. This has been tested (for a number of varieties) by obtaining seed from many single plants and testing the resistance of these single plant progenies to a single clone of *F. oxysporum* f. *lini*. Results of inoculation of such progenies of the 3 varieties, Dakota, Bison, and B 5128, as compared with bulk seed of Punjab 47, are in Table 3. The variety Dakota shows a great range of susceptibility with 90 of the 114 progenies falling in the 81–100 per cent disease class, 12 in the 0–5 per cent class, and the remaining 12 scattered between these 2 classes. The Bison progenies showed the least variation in regard to resistance. Punjab 47 (bulk seed) showed no resistance to the disease. Such heterogeniety indicates the necessity of working with single genotypic lines of the host as well as the pathogen, if any great degree of exactness is to be expected in testing for pathogenic races of the organism. Also in breeding for resistance or studying the mode of inheritance of resistance it is necessary to know the progeny reaction of each individual parent plant in order to explain the results.

TABLE 3.—*Progenies of single plant selections of 3 commercial flax varieties and plants from bulk seed of 1 variety classified for resistance to Fusarium wilt in greenhouse tests*

Variety	Number of progenies	Number of progenies in wilt percentage class: 0-5	6-20	21-40	41-60	61-80	81-100
Dakota	114	12	5	0	1	6	90
Bison	30	0	0	7	13	10	0
B 5128	50	8	19	13	8	0	2
Punjab 47	46*	0	0	0	0	0	46

* Not single plant progenies but 46 rows of bulk seed.

DISCUSSION.—Although a great deal of information concerning Fusarium wilt of flax has been published over a period of 50 years there is still no good explanation of the inheritance of resistance. Neither the number nor dominance of the genetic factors governing resistance have been determined in precise terms. One of the greatest difficulties encountered in tests for inheritance determinations lies in the fact that within nearly all wilt-resistant varieties or selections marked differences can be found between single plant progenies with regard to their resistance. In other words, marked heterogeneity of genotypes in this respect is the rule rather than the exception. When crosses are made between random plants of a wilt-resistant and those of a susceptible variety or between random plants of 2 resistant varieties subsequent generations will show considerable variation between the individual crosses with regard to their segregation into resistant and susceptible plants.

The other major factor that must be considered in a study of inheritance is the variability shown by the causal organism. Based upon their relative pathogenicity on a number of flax varieties pathogenic races have been shown to exist. This fact is apparent in nature as well as in artificial inoculations. Comparable varietal plantings in several naturally infested field plots often show a marked difference in the wilt reaction of the same variety in different plots or even within the same plot from year to year. This is true even in plots in the same immediate area; consequently, field tests generally cannot be reliable in determining the exact mode of inheritance. Also, the control of

environment is not possible in such field plots.

To determine the nature of inheritance it appears necessary to have parental lines of flax genotypically pure with reference to their wilt reaction and to use pure, natural clones of the pathogen. Inheritance of resistance can then be studied with any single clone of the organism. This can only be accomplished by using a uniform, artificial method of inoculation under a constant, ideal environment for disease development. Likewise, it is true that definite pathogenic races of the wilt fungus can be determined only when flax varieties homogeneous with reference to wilt reaction are used as differentials and are inoculated with single clones of the organism.

SUMMARY

A method of artificial inoculation of flax with Fusarium wilt was devised for use in the greenhouse to aid in the development of resistant varieties adapted in California and for use in fundamental studies of the organism and disease development. The method of inoculation involves the application of a uniform spore suspension to the seeds and bottom of the furrow after placing the seeds but before covering them with soil. Problems arising in greenhouse tests were the choice of temperature and soil type, insects or other fungi pathogenic to flax seedlings, and the maintenance of the natural clones of the pathogen. It was found that soil type may have a profound effect upon disease development. Clonal lines of the pathogen were maintained by selecting the natural type from single-spore transfers supplemented with frequent re-isolations from inoculated plants. Pathogenic races of the fungus were found to occur in California, based upon their relative pathogenicity upon 6 flax varieties. Considerable variation in resistance of single plant progencies was found to occur in a number of varieties, indicating the necessity of knowing the wilt reaction of progeny from each individual plant used in crosses for resistant variety development or in studying inheritance of resistance.

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THE CONTROL OF LEAF AND STEM RUST OF WHEAT WITH CHEMOTHERAPEUTANTS [1]

J. E. Livingston

The control of the various rusts of small grains with fungicides has never been considered practical even though dusting sulfur has been shown to be effective (2, 3). However, with the advent of aerial sprayers and with the examples of the effective application of herbicidal chemicals from the air, the feasibility of controlling rusts of cereals by chemical treatment appears more promising. Dickson et al (1) have shown that the development of effective chemotherapeutants for the control of stem rust is possible. If such a chemical could be applied from the air, the application would be rapid, without mechanical injury to the crop, and inexpensive, provided the chemical is such that a substantial area could be covered per load. A project was initiated to find or synthesize a chemotherapeutant suitable for aerial application and having the following characteristics: 1) will be absorbed and transported through the tissues; 2) will eliminate rust infections; 3) will prevent additional infection; 4) can be applied to plants at low volumes of not more than 5 gal. per acre; 5) will be non-toxic to plants.

MATERIALS AND METHODS.—In preliminary studies, results were similar with leaf rust, *Puccinia rubigo-vera tritici* (Eriks.) Carleton and stem rust, *P. graminis tritici* Eriks. and Henn. Since leaf rust is more readily cultivated in the greenhouse than stem rust on wheat, it was used as the principal test organism.

Inoculation was accomplished by diluting urediospores of *P. rubigo-vera* or *P. graminis tritici* with talc in a cyclone apparatus, described by Tervet (7), and dusting the mixture on 2-week-old seedlings of Cheyenne winter wheat. Inoculated plants were incubated in an illuminated moist chamber for 24 hours at 70° F., then returned to the greenhouse for 96 hours and treated. The greenhouse was maintained at 65° F. except in late spring when daytime temperatures frequently rose as high as 80° F.

Treatments were made by spraying the chemicals on the plants with a Devilbus atomizer until run-off occurred. Air pressure was supplied by an electrical pump. Triton X-45 wetting agent was added to all treatments at a concentration of 0.1 per cent.

[1] Accepted for publication May 8, 1953.

Published with the approval of the Director as paper No. 603; Journal Series, Nebraska Agricultural Experiment Station.

The author is indebted to Mr. Neal Shafer and Mr. Deane Finnerty for making the necessary arrangements for the aerial applications of the fungicides, to the Research Council of the University of Nebraska for financial support for the project, and to Dr. Hamilton of the Department of Chemistry for supplying many of the chemicals tested. He is also most grateful to Dr. Wellhausen, Local Director of the Agricultural Program for the Rockefeller Foundation in Mexico, and his associate, Dr. Gibler, for their cooperation in arranging for, and assisting with the field applications of the fungicides in Mexico.

Field trials made at Lincoln, Nebraska, involved applying calcium sulfamate, phenylhydrazine base, and sulfanilimide to wheat from an airplane. An Aeronca monoplane was equipped with a spray boom with 46 whirljet nozzles of body size number 5 and cap number 3, spaced 7 in. apart. The propellor driven centrifugal pump had a 1 in. outlet with an output capacity of 25-30 gal. per minute. A swath 36 ft. wide was sprayed at the rate of 5 gal. per acre. The plane flew 70-75 m.p.h. with the spray boom approximately 4 ft. above the wheat heads in order to minimize drift.

Calcium sulfamate was applied at the rates of 15 and 7.5 lb. per acre, phenylhydrazine base was applied as a 10 and 5 per cent solution, and sulfanilimide at the rate of 840 gm. per acre. Since sulfanilimide is only slightly soluble in water it was necessary to add 8 lb. of acetone to sufficient water to make a total of 5 gal. Triton X-45 wetting agent was used at the rate of 19 ml. per 5 gal. of solution. Three replications each ¼ acre in size were treated with each chemical.

Field trials were also conducted on Baart wheat near Toluca, Mexico, during August, 1952, in cooperation with the Rockefeller Foundation. Calcium sulfamate was applied at the rates of 10 and 15 lb. per acre, phenylhydrazine base was applied as a 2 and 0.5 per cent by volume, and Actidione was applied at the rate of 50 p.p.m., all in water solution. The chemicals were applied to wheat with a knapsack sprayer at the rate of 5 gal. per acre. Three plots, 25 yd. x 3 yd., with 3 replications each were sprayed with each chemical. One replicated plot received a single application, the second received 2 applications 14 days apart, and the third plot received 3 applications at weekly intervals. The first application was made on August 14, 1952, when the wheat was pollinating. A heavy shower of rain fell within 3 hours after the first application and nearly every afternoon thereafter for a period of at least 5 weeks. This provided excellent conditions for stem rust development and a severe test for the chemical treatments.

Results were recorded by comparing the number and size of pustules developing on treated plants with those on the checks. A rating scale of 0 to 5 was used with 5 indicating the maximum number of large pustules.

RESULTS.—Of 179 chemicals tested, several arrested rust development when the plants were sprayed to the run-off stage in the greenhouse. When those that were effective in the greenhouse were applied to wheat in the field at low volumes some were unsatisfactory.

Sulfamates.—Calcium, ammonium, and sodium sulfamate were very effective in stopping the development of rust pustules on wheat in greenhouse tests. The

margin of safety on wheat was greatest with the calcium salt and least with the ammonium salt.

Calcium sulfamate almost completely stopped the development of pustules of leaf rust following aerial application to Cheyenne winter wheat in the field at Lincoln, Nebraska, at the rate of 15 lb. per acre. In the treated areas, no flag leaf was observed with more than 5 per cent rust while those in the untreated areas showed a severity reading of 40-50 per cent with 100 per cent prevalence. Control was slightly less with the application of 7.5 lb. per acre.

Twenty-four hours after treatment the sprayed plants were slightly lighter in color then the unsprayed; however, at harvest the sprayed areas were darker than the untreated. No adverse effect of the treatment could be detected on the plants in the field. Yields in the treated plots were as high as or higher than those in the untreated. However, it is difficult to interpret the yield data since hot winds dried most of the leaves of the plants 1 week after treatment. The treated plants remained greener and did not lodge as badly as the untreated following the hot, dry winds. Obviously, the calcium sulfamate disturbs the physiological processes of the wheat plants but as yet there is no indication that this disturbance is detrimental.

Calcium sulfamate at 15 lb. per acre was very effective against stem rust on Baart wheat near Toluca, Mexico. At the time of treatment the average stem rust infection at the base of the plants was 65/80; on the sheath of the flag leaf it was 5–10/75. Twelve days after a single application all the pustules, even at the base of the plants, appeared to be dead. Wheat seedlings in the greenhouse were inoculated with urediospores from these plants and no infection was obtained. The untreated plants showed a stem rust reading of 30/80 on the sheath of the flag leaf and urediospores from these plants produced good infection on seedlings in the greenhouse. This was a relatively severe test since it rained approximately 3 hours after the application and nearly every afternoon thereafter.

At harvest time, 8 weeks after treatment, the single application of calcium sulfamate gave fair control of stem rust. With fortnightly and weekly applications, good control was obtained.

Applications of 10 lb. per acre gave good control of stem rust when applied fortnightly and weekly. The single application resulted in poor control although some grain was harvested.

The untreated plants in the field were all killed by stem rust and subsequently plowed under without being harvested.

Some reduction in the germination of seed from the aerial treated plants at Lincoln, Nebraska, was obtained. The percentage reduction is related to the amount of calcium sulfamate applied and the importance of this factor is dependent upon the concentration of the chemical necessary to control rust. This can only be determined by further field trials.

Hydrazines and related compounds.—Phenylhydrazine and several closely related compounds gave excellent control of the rusts when applied to the run-off stage in greenhouse tests. This group of chemicals appeared quite promising because most of the compounds tested were nonphytotoxic at an effective concentration. For example, phenylhydrazine base inhibited the development of *P. rubigo-vera* at a concentration of 0.5 per cent by volume and did not injure wheat seedlings at concentrations below 2 per cent. However, when used in the field with low volume applications phenylhydrazine base was disappointing. Twelve hours after aerial application of a 10 per cent concentration at Lincoln, Nebraska, white dead areas were prominent on the first and second leaves from the top of the plant and also on the stem. Development of rust was inhibited within a radius of ½ in. of the necrotic lesions but was not affected beyond this area.

Somewhat less rust developed in the field on flag leaves sprayed with a 5 per cent concentration. There was less injury and consequently the chemical may have been transported a greater distance through the tissues.

Concentrations of 2 and 0.5 per cent were used near Toluca, Mexico. Three weeks after the first treatment the fortnightly and weekly applications appeared to retard stem rust development. However, at harvest time no control could be detected.

Actidione.—The action of the antibiotic Actidione in greenhouse tests was very similar to that of calcium sulfamate. Against stem rust in the field in Mexico it gave some reduction in number of pustules 3 weeks after the first application. However, at harvest no control could be detected. It is probable that the concentration of 50 p.p.m. of the chemical used in the field was too low.

In greenhouse tests Actidione showed a very narrow range between the concentration that gave effective rust control and that which was phytotoxic.

Other compounds.—The sulfa-type compounds were of some value in reducing the number and size of pustules of leaf rust on wheat; however, only sulfanilic acid and 4-carbomethylaminophenyl sodium sulfonate, of the compounds tested, deserve further consideration. Sulfanilimide applied to wheat in the field at Lincoln with the airplane retarded development of leaf rust. The low solubility of the chemical, however, decreases the possibility of obtaining an effective concentration for aerial application.

Several compounds containing a mercapto-group or a naphthylamine-group caused a marked retardation in the development of rust on wheat seedlings.

The diethanolamine salt of maleic hydrazide produced an interesting reaction. Slight stunting of the plants occurred with a concentration of 0.1 per cent. Stunting became more pronounced with increasing concentration. The intensity of green likewise was increased. However, the unexpected result was an increase in the size of the pustules of leaf rust. Apparently photosynthetic activities continue and carbohydrates accumulate providing a favorable nutritional condition for rust development, even though cell

TABLE 1.—*Effect of pH on chemotherapeutic activity of 3 compounds to P. rubigo-vera on Cheyenne wheat seedlings*

Material	pH	Conc.	Injury [a]	Rust [b]
Actidione	4.0	50 p.p.m.	3	3
	5.0		3	3
	6.0		1	3
	7.0		0	5
	8.0		0	5
Phenylhydrazine HCl	4.0	0.5%	1	0
	5.0		0	0
	6.0		0	1
	7.0		1	1
	8.0		4	0
Calcium sulfamate	4.0	2%	3	1
	5.0		0	2
	6.0		0	2
	7.0		0	2
	8.0		0	2

[a] 0—no injury; 5—plants killed.
[b] 0—development of pustules completely suppressed; 5—development of maximum number of large pustules.

division and elongation are retarded (4). McIllrath (5) has shown that maleic hydrazide interferes with the translocation of carbohydrates and results in their accumulation in cotton plants. Naylor (6) showed the presence of a high concentration of sucrose in corn treated with maleic hydrazide.

The manganous and cupric salts of maleic hydrazide did not produce the phytotoxicity obtained with maleic hydrazide. The manganous salt caused some reduction in the development of the rust at a 5 per cent concentration.

Effect of pH on chemotherapeutic activity.—There was a great variation in the pH of the various chemical preparations tested. Several of the more effective preparations had a pH of 3.0 or lower. To determine whether pH would affect the rust inhibiting activity of certain chemicals, Actidione, phenylhydrazine HCl, and calcium sulfamate in water solution were adjusted to pH 4.0, 5.0, 6.0, 7.0, and 8.0 with 0.1 N HCl and 0.1 N NaOH, and sprayed on Cheyenne wheat seedlings 72 hours after inoculation. Triton X-45 was used as a wetting agent. Results are in Table 1. Check plants sprayed with water solutions at the various pH levels under test were not injured by the concentrations of alkali or acid required.

The best control and the greatest phytotoxicity with Actidione and calcium sulfamate occurred at the lower pH values. However, with phenylhydrazine HCl, severe injury occurred at pH 8.0 with only very slight injury at pH 7.0 and 4.0. There is, therefore, a definite relationship between the pH of the solution and its toxicity.

DISCUSSION.—Chemotherapeutants, to be useful in the control of stem rust of small grains, must have characteristics that will permit aerial application. Of primary importance is solubility in a noncombustible, nonphytotoxic liquid. Many of the chemicals used in the experiments reported here required either acetone, alkali, or acid as a solvent. Those requiring an acid solution were of little value because the amount of acid required was usually phytotoxic. However, wheat seedlings are quite tolerant to acetone and NaOH.

A systemic chemical is essential since with aerial application the droplets of spray are deposited only on the upper leaves of the plant in fields with good stands. This was observed with phenylhydrazine following aerial application to wheat. Numerous white necrotic spots appeared on the upper leaves within 24 hours. A few were observed on the second leaf from the top but only an occasional spot on the lower leaves. It is possible that a few very small droplets fell on the lower leaves without producing visible injury. However, pattern studies by Mr. Shafer, with the plane used in these tests, showed that approximately 75 per cent of the total volume falling on a glass slide when the plane was applying 5 gal. per acre fell in droplets varying between 1500 and 2000 mμ. in diameter. Therefore, the number of droplets that might fall on the lower portion of a plant would represent only a very minor portion of the total volume applied. Since stem rust frequently develops first and most abundantly on the lower portion of the stem it is necessary that the chemical be transported from the upper leaves to this region.

It is also desirable to have a chemical that will both eradicate the organism and protect the plant against reinfection. This will permit delaying the first application until there is the certainty that rust would otherwise develop. One application will then stop the development of existing infections and prevent new infections.

Calcium sulfamate meets most of these requirements. It is soluble in water in effective concentrations, it is either systemic or induces a systemic reaction in the plant, it will stop the development of the rust fungus, and it reduces the viability of spores in open pustules at the time of spraying. In experiments thus far it has shown only limited ability to impart immunity to the plants. This is a distinct disadvantage, but in most seasons it can be offset by an additional application. Under normal conditions in Nebraska the period from pollination to physiological maturity is 28 days. Stem rust appears about the time wheat is heading and each generation requires 10–14 days for development. Since the first application is made after rust development is initiated there is little likelihood that more than 1 additional application will be necessary. This places aerial spraying with chemicals that are either systemic or that induce a systemic action within the realm of practicability.

SUMMARY

One hundred and seventy-nine chemicals were tested to determine their value as chemotherapeutic agents in the control of *Puccinia rubigo-vera* on wheat.

Calcium sulfamate was the most effective chemical for stopping the development of pustules of *P. rubigo-vera* and *P. graminis tritici* in the tissue of wheat leaves in field trials. It was satisfactory for aerial application at low volumes of 5 gal. per acre in water

solution. Physiological changes were induced in wheat plants but in tests thus far the only detrimental effect detected was a slight reduction in the germination of seed from treated plants.

Actidione requires additional field tests. It appears to have some value both as an eradicant and in imparting immunity to wheat plants. However, the range of safety between fungicidal effectiveness and phytotoxicity is narrow.

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THE EFFECT OF SOIL MOISTURE ON THE GROWTH AND SURVIVAL OF FUSARIUM OXYSPORUM F. CUBENSE IN THE LABORATORY [1]

R. H. Stover [2]

The use of flood fallowing (5,6) to reclaim soil infested with the Panama Disease fungus has focused attention on the soil moisture relationships of *Fusarium oxysporum* f. *cubense* (E.F.S.) Sny. and Hans. In the first paper of this series (6), the effect of flooding on the general fungus and Fusaria flora of clay loam soils was described. Soil moisture is the environmental factor over which some control can be exerted through flood fallowing and irrigation practices. Using the facilities available for irrigation and flooding, it may be possible to manipulate soil moisture content to the detriment of the fungus. Before experiments could be undertaken with this object in mind, it was necessary to obtain some information as to the effect of different soil moisture contents on fungus growth and survival. Accordingly, preliminary laboratory experiments were performed, and the results are reported in this paper.

Relatively little is known about the effect of soil environment on Panama Disease or *F. oxysporum* f. *cubense*. When this disease appeared in Honduras, light textured soils with an acid reaction were rapidly invaded in contrast to certain loam soils where disease spread was slow and irregular. From 1929 to 1932 the Research Department of the United Fruit Company studied the correlation between disease spread and soil characteristics such as texture and pH (7). Although many alkaline heavy textured soils were "resistant" to disease spread, there were some noteworthy exceptions. Wardlaw (8) obtained some evidence from pot experiments indicating that infection of banana roots was favored by fluctuating moisture conditions and carbon dioxide vitiation. In fact he did not obtain infection in soil that was constantly wet or dry. Hollis (1) found that *F. oxysporum* f. *cubense* had considerable tolerance to low oxygen tensions and high carbon dioxide content. It survived 13 weeks in sealed cultures. Meredith (3) showed that this fungus grew more rapidly in sandy loam than in silt and clay loams. Light textured soils in Central America contained more of this *Fusarium* than did clay soils (4). In a preliminary report (5) it was stated that growth and survival of *F. oxysporum* f. *cubense* was optimum in soils containing a moisture content of 25 per cent saturation.

METHODS.—The soils used (Table 1) were air dried, screened, and with one exception not sterilized. Cultures for infesting soil were prepared from 10 per cent by volume of corn meal in sand. After 2–4 weeks of fungus growth in this substrate, portions were thoroughly mixed with the soils under study. Approximately 200–250 gm. of the infested soil was then placed in glass tumblers and brought to various percentages of moisture saturation by adding measured amounts of water. Moisture levels of 0, 25, 50, 75, and 100 per cent saturation were maintained within 10 per cent of the desired level by frequently weighing and adding moisture lost by evaporation. As the soil at 25 and 50 per cent moisture levels was not mixed after the addition of water a variation of 10 per cent existed between the top and center of the tumbler 1 day after the addition of water. In certain experiments the soil was submerged under 1 in. of water. To reduce evaporation from the surface of the soil, all tumblers were covered with petri dish tops. The tumblers were stored at room temperature which varied from 70° to 97°F.

Platings were made by taking 1 gm. of soil from near the center of the tumbler with a narrow bladed knife. This sample was added to 99 ml. of sterile water, and after shaking 2 minutes, 10 ml. were transferred to 90 ml. of water to give a final dilution of 1:1000. Three plates with 1 ml. and 3 with .5 ml. of

[1] Accepted for publication May 4, 1953.
[2] The writer is indebted to Drs. N. C. Thornton and V. C. Dunlap for their co-operation and assistance and to the staff members who offered helpful suggestions.

TABLE 1. *Characteristics of soils used in moisture experiments with Fusarium oxysporum f. cubense*

Soil	Percentage of Clay	Silt	Sand	pH	Source	Panama Disease History
Loam	18	46	36	8.4	Mopala Farm, Honduras	Slow, irregular spread of disease
Sandy loam	6	18	76	6.8	San Alejo, Honduras	Rapid spread, area abandoned for banana production
Very fine sandy loam	8	40	52	7.0	Sevilla, Colombia	Disease has not been found in this area

the 1:1000 dilution were poured for each sample using peptone dextrose agar (2). All figures are given on the basis of *F. oxysporum* f. *cubense* per gm. of air dry soil.

In experiment 7, pieces of fresh banana roots approximately 5 mm. in diameter and 7–12 cm. long were placed in each tumbler of soil. After 258 days the partially decayed roots were removed, washed, placed in 100 ml. of water, ground in a Waring blendor for 1 minute, and plated at a dilution of 1:1000. Ten ml. of the root-water suspension were air-dried and the final fungus counts were given on the basis of fungi per gm. of air-dry roots. Treatment with the Waring blendor would tend to increase the fungi appearing on the plate by fragmentation of mycelium. Nevertheless, it does give an approximation of the amount of *Fusarium* in and on the roots. Soil from around the root pieces was plated as described above. At 75 and 100 per cent saturation the roots in the loam soil were completely decayed and could not be removed.

EXPERIMENTAL RESULTS.—The results of experiment 1 (Fig. 1 and 2) show the effect of soil moisture on fungus growth in Mopala loam and San Alejo sandy loam soils. The initially large *Fusarium* population at moisture levels above 25 per cent saturation decreased sharply during the first 15 days after the soil was infested. This population decline continued for 60–80 days at a progressively slower rate, and then levelled off to populations related directly to soil moisture content; large populations were maintained at lower moisture levels. The initial decline was slower at moisture levels of 25 and 50 per cent saturation than at 75 and 100 per cent, and occurred later. Under submerged conditions the fungus did not survive 40 days. At saturation, however, survival was 7 times as long in sandy loam as in loam. Otherwise, population changes in the 2 soils were not significantly different. Apparently, constant moisture levels of 25 per cent saturation were most favorable to growth and survival of *Fusarium*.

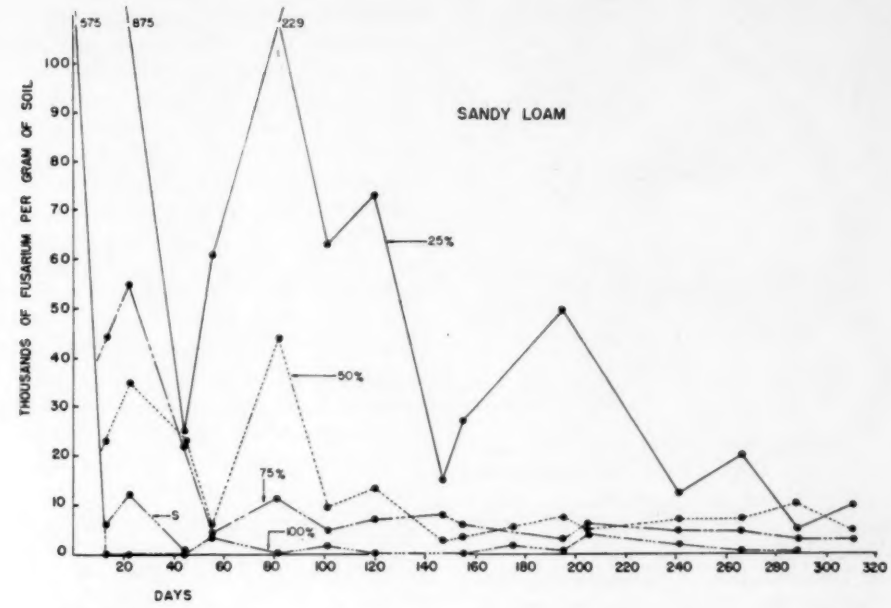
Similar results were obtained in several replications of the above experiment.

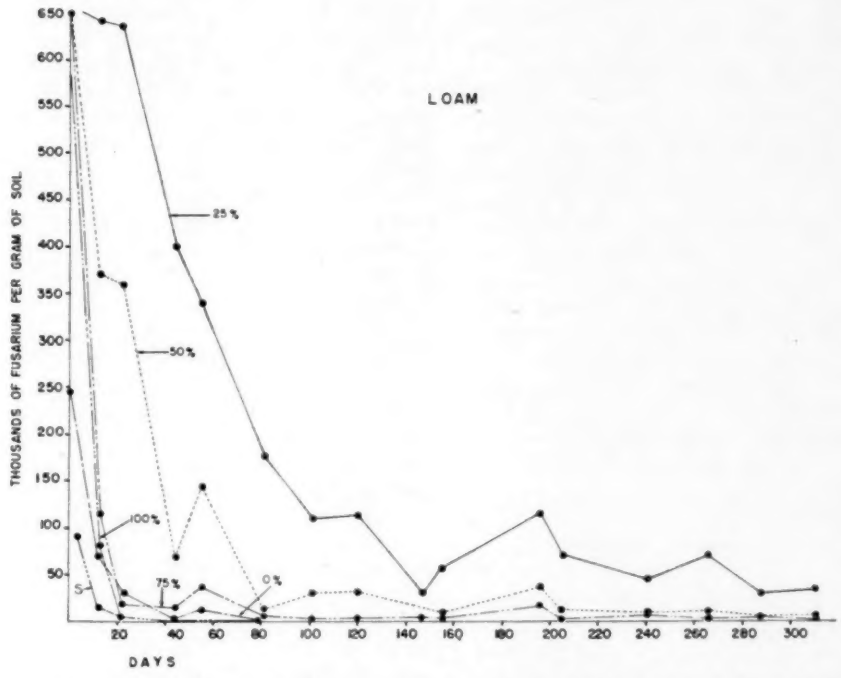
In experiment 2, one portion of the loam used had been submerged *in situ* 4 months under 4 ft. of water. As shown in Fig. 3, 4 months submergence had considerable effect on the subsequent soil moisture relationships of *Fusarium*. Higher moisture levels in previously submerged soil had less effect on the fungus than in non-submerged soil. Thus, population differences in the range of 25–50 per cent saturation were less apparent in previously submerged soil. Growth and survival of the fungus at moisture levels from 25–75 per cent were greater in previously submerged soils than in non-submerged soil. In fact, for certain periods, growth at 50–75 per cent saturation in previously submerged soil was greater than that at 25 per cent saturation in non-submerged soil. The long period of fungus survival in saturated soil previously submerged, is contrary to results using non-submerged soil.

The loam soil used in experiment 3 was previously submerged as in experiment 2. However, 1 portion of this soil was sterilized and *Fusarium* growth in it was compared with that in the non-sterile portion. Again, growth differences in the middle moisture range were less apparent in both soils than in non-submerged soil (Fig. 4). However, in most cases, growth was greater at 25 per cent saturation than at higher moisture levels. As expected, growth at 25–75 per cent saturation was greater in sterilized soil than in non-sterilized soil at 25 per cent saturation. In both sterilized and non-sterilized submerged soils, survival at saturation was much longer than in corresponding soils not sterilized or submerged.

In the above experiment at moisture levels from 25–100 per cent saturation, *Fusarium* growth and survival in different soils was greatest in the following order: loam, previously submerged then sterilized; loam, previously submerged; sandy loam; and loam. Apparently, submerging or sterilizing reduced the magnitude of the growth differences at the moisture levels used and increased the survival of the fungus at all soil moisture levels.

In experiment 4 an organic soil from the surface of a drained flood fallowed area and the Mopala loam were compared at 2 moisture levels: 25 and 50–75 per cent saturation. Although growth of *Fusarium* was greater in both soils at 25 per cent saturation, there was no significant difference between growth in the organic soil previously submerged and in Mopala loam. This seems contrary to our previous results (experiment 2) with submerged soil. However, the previously submerged loam in experiments 3 and 4 was removed from the lake floor while covered with 4 ft. of water. At the time of removal, the fungus population was 4–5 thousand per gm. of air dry soil. The organic soil in experiment 4 was taken from the surface of a floor fallowed lake 3 weeks after the lake was drained. The fungus population of this soil varied from 50–100 thousand per gm. At least 1/3 of these



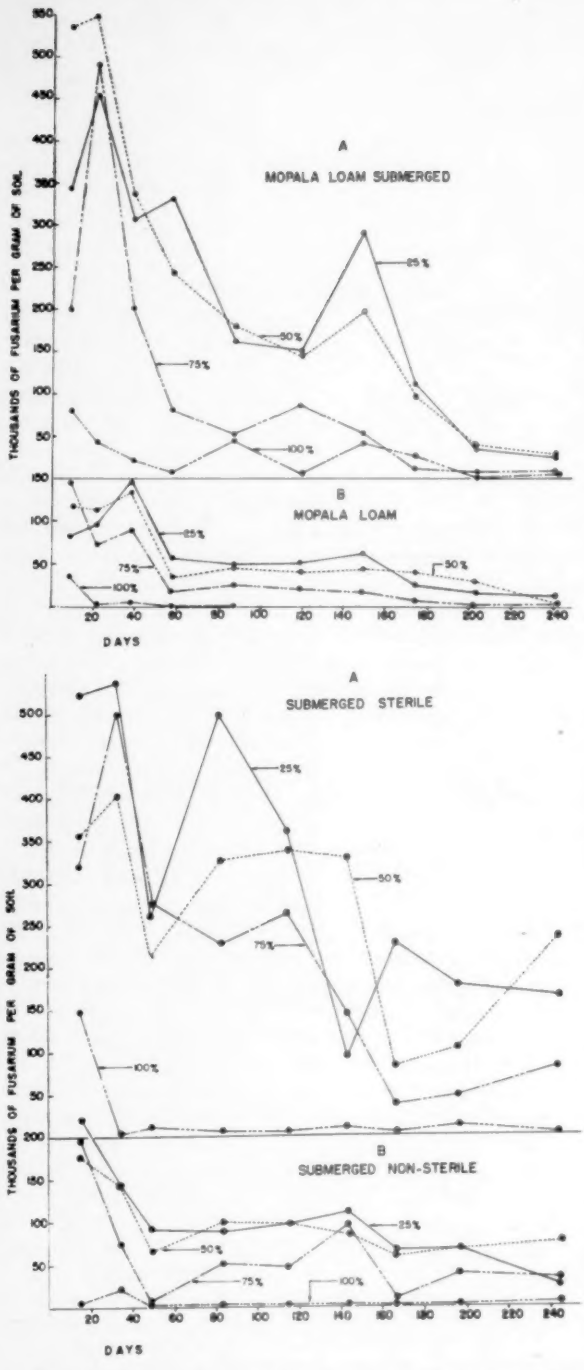


FIGS. 1–2. Fig. 1. The effect of soil moisture on the *Fusarium* flora of San Alejo sandy loam in experiment 1; moisture levels were maintained at 0, 25, 50, 75, and 100% saturation. Series (s) was submerged under 1 in. of water.—Fig. 2. The effect of soil moisture on the *Fusarium* flora of Mopala loam in experiment 1. Moisture levels were maintained at 0, 25, 50, 75 and 100% saturation. Series (s) was submerged under 1 in. of water.

fungi were *Trichoderma* species. It is believed that the large fungus population of the organic soil eliminated the differences in *Fusarium* growth between soils that received such widely different treatments.

The results of experiment 5 are not presented as they were similar to those of experiment 1. The behavior of the fungus in very fine sandy loam from Sevilla, Colombia, where Panama Disease has not appeared, was similar to that in Mopala loam from Honduras.

In experiment 6, 3 soils were infested with *Fusarium* and subjected to the variable moisture conditions of outdoor weather. Changes in the fungus and *Fusarium* populations were followed and these are shown in Ta-



FIGS. 3–4. Fig. 3. The effect of soil moisture on the *Fusarium* flora of (A) Mopala loam previously submerged and (B) Mopala loam non-submerged in experiment 3.—Fig. 4. The effect of soil moisture in experiment 4 on the *Fusarium* flora of (A) Mopala loam previously submerged and sterilized and (B) Mopala loam submerged and non-sterilized.

ble 2. In sandy loam soil, a sharp decrease in the *Fusarium* population occurred between the 23rd and 46th day. In the compost and clay loam soils, a sharp population decline occurred between 46 and 72 days. After these first recessions in the *Fusarium* population, a subsequent population decline occurred in the next 30 days. After this last recession, the *Fusarium* population remained at a relatively low level. These *Fusarium* population recessions were accompanied by corresponding increases of other soil fungi, the largest single component of which were species of *Aspergillus* and *Penicillium*. The sandy loam soil had initially a much larger population of *Aspergillus* and *Penicillium* species than the loam or compost soils. This may have accounted for the earlier *Fusarium* recession in sandy loam soil. Floral changes could not be correlated with changes in soil moisture content. Once the *Fusarium* recession began, soil moisture content appeared to have little or no direct affect on its course. Evidently, a fluctuating environment such as that present under field conditions is less favorable to the growth and survival of *Fusarium* than conditions in the laboratory where moisture levels were controlled. Nevertheless, soils in the above experiment were free of all plant growth, a condition that rarely exists for long in banana plantations.

In experiment 7, banana roots were added to the soil. An abundant *Fusarium* population was found in the dead and largely decayed roots after 258 days (Table 3). Generally, at all moisture levels, there was a greater abundance of *Fusarium* in the dead and partially decayed roots than in the surrounding soil. Also, the fungus was present in considerable abundance in roots in near-saturated soil. In addition to *Fusarium*, there was a large fungus population present in the decaying roots when contrasted with the numbers present in the soil. There were not sufficient data to determine the effect of soil moisture on root colonization. Therefore, further experiments are underway to obtain more information.

DISCUSSION.—The effect of moisture on fungus growth was most evident in non-sterile soils. How much of this effect was caused by moisture acting indirectly through soil aeration and changing the soil flora is not known. In sterile saturated soil, a rapid recession in the *Fusarium* population occurred. This can be attributed mainly to the direct effect of moisture on the fungus or lack of aeration rather than to the action of the soil flora. Nevertheless, the soil flora influences survival at these high moisture levels as indicated by longer survival in sterile saturated soil than in similar soils non-sterilized. The importance of the soil flora is indicated in the range of 25–75 per cent saturation where, in non-sterile soil, population differences between soils of different moisture content are pronounced. In sterile soil, however, growth at 75 per cent saturation often is greater than that at 25 per cent in non-sterile soil. Also, the differences in growth between 25, 50 and 75 per cent saturation are less pronounced in non-sterile soil.

Submerging soil for several months prior to use had an effect similar to sterilization but of lesser magnitude. Thus, in previously submerged soils fungus sur-

TABLE 2. *Changes in the fungus and Fusarium flora of three soils exposed to outdoor climate in partially buried pots (Experiment 7)*

Days After Adding Inocula	Compost Mixture		Loam[a]		Sandy Loam[a]	
	Fusarium[b]	Fungi[b]	Fusarium	Fungi	Fusarium	Fungi
0	223,000	283,000	93,200	——[c]	41,000	319,800
18	206,000	——	204,000	——	202,000	——
23	746,230	——	676,000	——	308,000	——
46	177,120	110,160	286,400	2,000	54,000	80,600
72	15,600	——[d]	54,790	45,210	2,500	67,600
100	600	97,000	2,060	111,000	1,310	176,450
105	12,840	77,160	1,020	102,000	2,060	94,760
135	310	97,000	810	97,110	1,220	99,530
146	0	122,690	4,280	149,800	0	122,660

[a] For characteristics of these soils see Table 1.
[b] *Fusarium oxysporum* f. *cubense* and other fungi per gm. of air dry soil.
[c] Other fungi not counted because of dominance of *Fusarium* colonies.
[d] Reading lost.

vival is longer at saturation, and greater in the range of 25–75 per cent saturation than in non-submerged soils. However, if a soil submerged several months is drained, and allowed to remain *in situ* for 3 weeks as in experiment 4, the situation is changed. During the period after draining, there is a period of increased fungus and bacterial growth (6). The soil becomes highly populated with fungi and in most cases, *Aspergillus, Penicillium* and *Trichoderma* species are predominant. These soil flora changes reduce the effect of previous flooding and the differences between previously submerged and non-submerged soil are soon eliminated.

A fluctuating environment such as occurs in nature is less favorable to *Fusarium* than the environment associated with constant moisture levels in the range of 25–75 per cent saturation. The soil placed outdoors was never wet for long and moisture from showers was soon evaporated by the hot sun and wind. Certain components of the soil-inhabiting fungi are more adaptable to these sharp fluctuations in soil moisture than *F. oxysporum* f. *cubense*. *Aspergillus* and *Penicillium* species, for example, are perhaps more adaptable in their requirements than *Fusarium* and these species are predominant among succession fungi as the *Fusarium* flora is declining. In addition to the rise of a competing flora probably better adapted to environmental change, exhaustion of food supply may play an important role in the *Fusarium* recession.

Indirect evidence indicating the relation of food supply to fungus recession was obtained in most experiments. During the first 7–14 days after infesting the soil, there was a 3–8-fold increase in *Fusarium* colonies developing on the plates. This increased fungus growth immediately preceded the rapid recession that inevitably followed. During this period of rapid growth, *Fusarium* dominated all fungi developing on the plates even though the soil may have had an initially high concentration of *Aspergillus* and other species. These high population levels are not indicated in the graphs which were drawn to show the effect of soil moisture on fungus growth after these temporarily high populations began to level off and population differences became more evident.

The rapidity of the recession immediately following the initial population rise indicates an exhaustion of available food. That the rise of a competitive fungus flora is not the main cause is shown by the fact that

TABLE 3. *Changes in the fungus and Fusarium flora of soils containing dead banana roots*

Days After Soil Infested	Substrate	Percentage Moisture Saturation of Soil							
		25		50		75		90–100	
		Fusarium[a]	Fungi[a]	Fusarium	Fungi	Fusarium	Fungi	Fusarium	Fungi
1	loam	135,000	——[b]	135,000	——	135,000	——	135,000	——
36	loam	144,000	432,800	104,600	489,000	120,000	86,400	0	26,000
66	loam	106,620	27,970	65,190	52,890	21,670	8,000	0	1,600
258	loam	107,200	387,360	37,270	86,470	9,880	134,470	——	——
258	roots in loam	217,000	2,161,200	881,000	——	roots decayed		roots decayed	
1	sandy loam	190,000	——	190,000	——	190,000	——	190,000	——
36	sandy loam	123,900	773,900	64,300	324,000	37,500	218,800	44,500	153,600
66	sandy loam	148,680	99,120	72,360	97,170	11,880	81,520	2,030	90,810
258	sandy loam	11,800	333,090	5,090	106,890	2,500	101,880	2,290	89,540
258	roots in sandy loam	57,200	2,400,000	289,200	1,355,100	606,600	1,014,000	61,600	209,000

[a] *Fusarium* and other fungi per gm. of air dry soil or roots.
[b] *Fusarium* colonies were dominant on plates, few other fungi present.

the increase in soil inhabiting fungi begins at the time of *Fusarium* recession. Also, this fungus was present in abundance in partially decayed banana roots that contained a large population of other fungi. It is likely that the bacterial flora contributed to the eventual exhaustion of the food supply in addition to the rapid growth of *Fusarium*. After the base food supply (10 per cent corn meal-sand) was exhausted, *Fusarium* began to recede and the stage was set for the succession of other soil inhabitants whose nutritional requirements are less exacting than those of *Fusarium*. At saturation, the available food could not be utilized by *Fusarium* and the recession began immediately. In previously submerged or sterilized soil, there were fewer competing organisms and the recession occurred later than in non-sterile soils.

Apparently this *Fusarium* can exist for long periods in soil at low population levels. Its survival for long periods may be related more to food supply than to the presence of competitors and antagonists. This is indicated by the greater population of *Fusarium* and other genera present in decayed banana roots after 9 months, than in the surrounding soil. Thus, where a suitable food supply such as dead banana roots is available, *F. oxysporum* f. *cubense* is capable of competing with other soil inhabitants.

SUMMARY

The effect of soil moisture on the growth and survival of *Fusarium oxysporum* f. *cubense* in different soils was studied.

In non-sterile soils and under the conditions of these experiments 25 per cent saturation was optimum for growth and survival.

As the soil moisture content was increased from 25 per cent saturation to saturation, fungus growth and survival decreased in non-sterile soils. At saturation, survival was longer in sandy loam than in loam and was greatest in sterile and previously submerged soil.

In sterilized soil the differences in growth and survival at 25, 50, and 75 per cent saturation were less than in non-sterile soil. Submerging soil under water for 4 months had an effect similar to sterilizing but of lesser magnitude. Fungus growth and survival was greater in previously submerged soil for a period of 200 days at 25, 50, and 75 per cent saturation than in non-sterile soil at 25 per cent saturation.

Fluctuating moisture levels under outdoor conditions were unfavorable to *F. oxysporum* f. *cubense* but favored the succession of other soil fungi.

The fungus colonized dead banana roots for a period of at least 9 months. Colonization occurred at soil moisture levels up to near saturation, and the relative abundance of *Fusarium* in partially decayed banana roots was considerably greater than in surrounding soil.

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THE PRODUCTION AND SURVIVAL OF SPORANGIA OF PHYTOPHTHORA INFESTANS ON TOMATO AND POTATO PLANTS IN THE FIELD [1]

J. R. Wallin [2]

Since its first appearance in Europe, *Phytophthora infestans*, (Mont.) De Bary, became known as the 'cool, wet weather' pathogen, probably because it reaches its maximum intensity on potato and tomato foliage during prolonged periods of cool, wet weather. However, an experience with potato late blight at Crystal Lake, Iowa, in the summer of 1948, indicated that blight epiphytotics develop under other conditions. At that time, in an unsprayed potato plot inoculated with *P. infestans*, late blight developed gradually and consumed the plants during an 8-week period of limited rainfall and intermittent daily high temperature maxima. During this period temperatures were below 75° F. and humidities above 90 per cent for 10 or more hours on at least 1 of every 7 nights. On the nights when these temperature-humidity conditions prevailed, the fungus sporulated on the lesions of the blighted potato leaflets. Moreover, the sporangia thus produced, if borne on the shaded under surfaces of the leaflets, remained viable throughout warm, dry afternoons following such evenings. These findings stimulated further field studies of the behavior of the fungus on the potato and tomato.

Studies on production and survival of *Phytophthora infestans* sporangia on blighted potato plants were made at Clear Lake, Iowa, in 1950 and 1952; and on blighted potatoes and tomatoes at Ames, Iowa, in 1951 and 1952. Results of the studies are presented here.

MATERIALS AND METHODS.—Studies at Clear Lake in 1950 were conducted in a plot of Cobbler potatoes inoculated with a potato isolate of *P. infestans*. Spore viability determinations were made from water mounts of sporangia in hollow ground slides placed in moist petri dishes and held at about 65°F. until indirect germination ceased. The percentage of viable sporangia was determined from germinated sporangia in 4 counts of 25–100 sporangia.

In 1952, observations at Clear Lake were made in a plot of Cobbler potatoes affected by late blight.

At Ames, Iowa, in 1951 observations on development and collections of sporangia were made in 2 plots. In plot 1, in which potato and tomato plants were planted alternately in the row, 2 potato hills had been inoculated with a potato isolate of the pathogen. In plot 2, similarly planted with both hosts, 2 tomato plants had been inoculated with a tomato isolate. Potato leaflets were collected from plot 1, and tomato leaflets were taken from plot 2. Sporangia viability was determined as described above except that the spores were incubated at 53.6° F. for 12 hours.

At Ames in 1952 similar studies were conducted in a potato-tomato plot in which the potatoes had been inoculated with a potato isolate.

Experimental results.—At Clear Lake on August 10, 1950, at 9 P.M., 10 diseased potato leaflets free of sporangia of *P. infestans* were tagged. For the next 11 hours, until 8 o'clock the following morning, the temperatures were below 70° F. and the relative humidities were above 90 per cent. By 8 A.M. sporangia were noted on all the tagged leaflets. The 10 leaflets were observed every hour until 5 P.M. By this time some of the sporangia had been discharged; apparently no more were produced.

On August 15, 1950, at 2 P.M., *P. infestans* was sporulating copiously on the surface of the lesions of shaded and unshaded leaflets. Ten of these blighted leaflets were brushed free of sporangia and tagged for observation. Ten hours later, no sporangia were evident on the lesions. These leaflets were observed the next day at 5 times.

Sporangia were present on 3 leaflets by 8 A.M. During 6 hours of the night the temperature was 70° F. or less and the humidity 90 per cent or more. By 11 A.M. the temperature was 89° while the relative humidity had dropped to 45 per cent. Nevertheless, spores had been produced on 5 leaflets. By 2 P.M. there were more sporangia on 7 leaflets. The heaviest sporulation occurred on the 3 shaded leaflets. These results indicated that sporangia developed during adverse daytime conditions.

In another attempt to determine the time of day at which viable late blight sporangia might be present, water mounts of sporangia were made from blighted potato leaflets collected in the morning, afternoon and evening. Results are in Table 1. Viable sporangia were found each time samples were taken. In some instances the percentage of viable sporangia was greater on bottom than on top leaves. During a day with an 88° F. temperature maximum, only a few viable sporangia were present on top or bottom leaflets. A most significant finding was the occurrence of viable sporangia on the leaflets at 8 and 12 P.M. following daytime temperature maxima in the 80's. This clearly demonstrated the capacity of the fungus to survive unfavorable conditions.

Another experiment was conducted to determine whether or not sporangia would develop by evening on infected potato leaflets brushed free of sporangia in the morning. After different time intervals the brushed leaflets were examined for spores.

Viable sporangia were produced after 9 hours on blighted potato leaflets but were more numerous after

[1] Accepted for publication May 13, 1953.
Journal paper no. J-2250 of the Iowa Agricultural Experiment Station, Ames, Project 1163. Report of an investigation conducted under the Research and Marketing Act of 1946 in cooperation with the Botany and Plant Pathology Section of the Iowa Agricultural Experiment station.

[2] Pathologist, U.S.D.A., B.P.I.S.A.E., Division of Mycology and Disease Survey.

TABLE 1.—*Viability of sporangia from potato leaflet lesions collected at several times on 4 days. Clear Lake, Iowa. 1952*

Date	Time	Sky cover	Dew	Temp.	Percentage viable sporangia on leaves Top	Mid	Bottom
7/31	8 AM	Clear	Yes	70	10[a]		62
	2 PM	"	No	88	7		3
	8 PM	"	Yes	65	1		3
8/1	8 AM	"	Yes	68	41		44
	2 PM	"	No	86	35		37
8/5	11 PM	"	Yes	51		8	
8/6	8 AM	"	Yes	66		27	
	11 AM	"	No	77	23		24
	2 PM	"	No	81	1		38
	4 PM	"	No	80	1		41
	8 PM	"	Yes	67		23	
	12 PM	"	Yes	57		20	
8/7	9 AM	"	Yes	77	14	26	24
	2 PM	"	No	82	14		52
	8 PM	"	Yes	67	19		16

[a] Each figure represents the mean of 100 spores counted for each of 10 leaflets or 1000 sporangia.

TABLE 2.—*The percentage of viable sporangia obtained at 6 times from late blight lesions of top, mid, and bottom leaflets brushed free of sporangia at 4 times*

Date	Hour leaflet		Temp.	Percent viable sporangia on leaflets from Top	Mid	Bottom
7/31	Brushed	10 AM	74			
	Picked	10 PM	60	5[a]		0
7/31	Brushed	2 PM	88			
8/1	Picked	2 PM	66	37		17
8/1	Brushed	3 PM	86			
	Picked	12 PM	70		45	
8/2	Picked	8 AM	76		64	
8/6	Brushed	11 AM	77			
	Picked	12 PM	57		11	
	Picked	9 AM	77	17		29

[a] Each figure represents the mean of 100 sporangia counted on each of 10 leaflets.

longer periods (Table 2).

Moreover, sporangia were produced on top leaves exposed to the sun. In addition, the data showed that blight lesions free of sporangia in the morning and early afternoon may display viable sporangia by 10 or 12 P.M. at the onset of favorable infection conditions.

At Ames, Iowa, in 1951, studies were conducted on the morning, afternoon, and evening production and viability of late blight sporangia on potato and tomato leaflets. On 6 mornings between 8 and 10:30 A.M. viable sporangia were obtained from blight lesions on potato leaflets. The spore viability per morning ranged from 4 to 60 per cent. On 3 mornings viable sporangia were collected from tomato leaflets. The viability ranged from 4 to 17 per cent. On 1 morning following a period of only 5 hours of concurrent temperature-humidity of 70° F. or less and 90 per cent or more, 4 per cent viable sporangia were collected from blight lesions on potato and tomato. On 3 afternoons at 1:30, 7, 13 and 39 per cent viable sporangia were collected from potato leaflet lesions when temperature maxima were 92°, 85°, and 86° F., respectively. During the same afternoons, 6, 7, and 37 per cent viable sporangia were obtained from tomato leaflets.

Viable sporangia were obtained from potato and tomato leaflet lesions on 5 of 6 evenings at 8 or 9 P.M. The highest percentage, 58 per cent, of viable spores from potato leaflets was obtained at 9 P.M. when the air temperature was 83° F. The highest percentages of viable sporangia, 54 and 55 per cent, were collected from tomato leaflets on 2 evenings at 8 P.M. when air temperatures were 74° and 68° F., respectively. At 8 P.M. on an evening following an afternoon air temperature maximum of 92° F., viable sporangia were not present on tomato or potato leaflet lesions.

In the 1952 trials, leaflets brushed free of sporangia

TABLE 3.—*Production and viability of Phytophthora infestans sporangia on potato leaflets at 4 times on 4 days*

Date	Leaflets brushed	*Leaflets collected* 6 P.M. Mn. % spores viable	6 P.M. Mn. no. spores scope field	8 P.M. Mn. % spores viable	8 P.M. Mn. no. spores scope field	10 P.M. Mn. % spores viable	10 P.M. Mn. no. spores scope field	12 P.M. Mn. % spores viable	12 P.M. Mn. no. spores scope field
8/19	8 AM	2[a]	6[b]	0	3	0.3	3	0	1
	10 AM	0	4	4	16	0.3	1	0.3	1
	12 AM	8	12	0.5	2	0	0.5	0	0.5
8/20	8 AM	0.7	0.5	0.3	1	2	1.5	8	5
	10 AM	0	0.3	0	1	0	1.5	9	16
	12 AM	0	0.5	4	3	44	21	23	28
8/21	8 AM	8	5	15	1.5	0	1	6	11
	10 AM	4	40	77	14	29	11	33	88
	12 AM	6	2.5	6	7	5	13	23	10
8/22	8 AM	45	28	14	27	38	110	72	1.5
	10 AM	10	6	57	121	36	53	44	170
	12 AM	25	4	23	31	12	2	80	1

[a] The mean percentage viable sporangia in 400. The number of viable sporangia in 100 was determined for each of 4 leaflets.

[b] The mean of the number of sporangia counted in 12 microscope fields, 3 per leaflet for 4 leaflets.

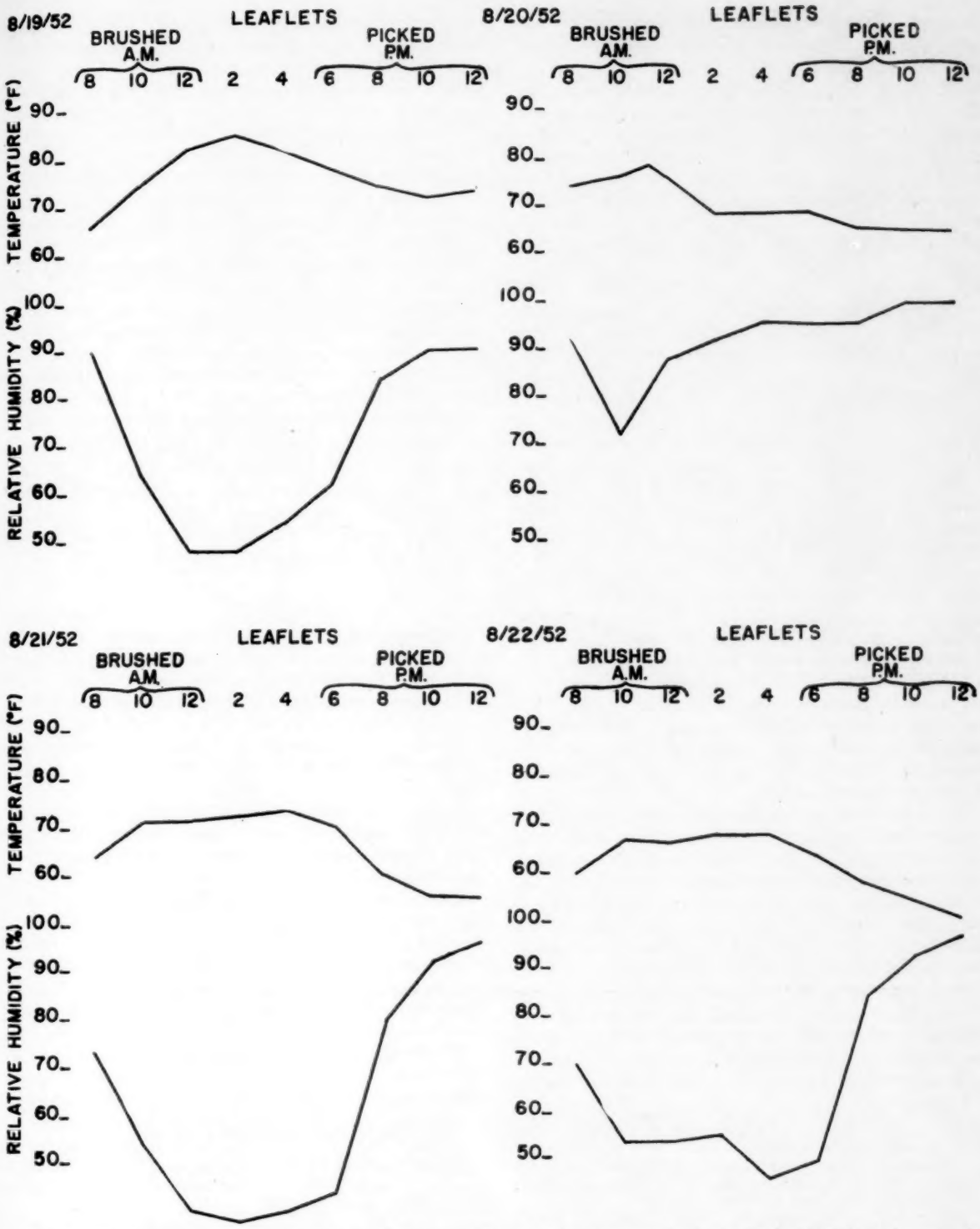

FIG. 1.—The temperatures and relative humidities prevailing at 3 times potato leaflets were brushed free of sporangia and 4 times that leaflets harvested and examined for sporangia.

at 3 times in the morning were observed for sporangia development at 4 times in the evening. Data are in Table 3.

Data in Table 3 were transformed into angles[3] and

[3] Snedecor, G. W. 1946. Statistical methods. Iowa State College Press, Ames, Iowa.

analyzed. Highly significant differences due to days were obtained. Differences due to brushing or picking were not significant.

Obviously, August 22 was much more favorable than the other 3 days for production and viability of sporangia. The appearance of viable sporangia on leaflets within 6 hours after they were brushed was particularly surprising. These results demonstrated that sporangia were formed during periods of the day usually considered adverse. However, the greatest number of viable sporangia were obtained on the days when the daily temperature maxima were 69° and 72° F., respectively.

Figure 1 shows the temperature and humidities that prevailed on 4 days from the time the lesions were brushed until they were picked. Low atmospheric humidities prevailed during 3 of the 4 days.

DISCUSSION.—*Phytophthora infestans* is known as the 'cool weather' fungus because the result of past researches have shown that 70° F. is the optimum and 75° the maximum for most of its infection processes, i.e., sporulation, germination, reinfection, and growth within the host. Sporulation occurs at temperatures from 37° to 79° with an optimum of 70°.[4] The optimum temperatures for indirect and direct sporangial germination are 53.6° and 75.2° respectively.[5] Swarmspore germination occurs over a wide range of temperatures from 37.4° to 82.4°.[4,5] Greatest germ tube elongation occurs at 72° [5] while the incubation period for the pathogen on potato foliage is the shortest, 66–77 hours at 73.4°.[4] Therefore, temperatures much over 75° must be assumed to be unfavorable for the foliar infection processes of the pathogen. High temperatures are especially unfavorable to the longevity of the sporangia, which rapidly lose their viability (ability to produce zoospores) at temperatures about 68°.[4,5]

P. infestans is known also as a 'wet weather' fungus because following Melhus's work most students of late blight have recognized that late blight epiphytotics follow periods of humid, wet weather. Furthermore, sporangia were shown to lose their viability at humidities less than 90 per cent.[4] Moreover, the following published statement [4] illustrates the supposed lethal effect of low humidity on the survival of *P. infestans* sporangia. "Unless sporania germinate during the night of their formation, they would not live through a warm, dry day nor would they survive on the upper leaves of plants during a cool, cloudy day."

Results obtained from the studies described herein indicated that on potato and tomato foliage in the field the late blight fungus can withstand lower humidities and higher temperatures than previously supposed. For example, spores survived long periods of seemingly unfavorable conditions, i.e., air temperature from 75° to 90° F. with concurrent humidities of 40 and 50 per cent. Furthermore, sporangia produced during 1 night survived adverse diurnal conditions until the next night. In some instances relatively high percentages of sporangia on the leaflets survived the so-called lethal air temperature of 80° to 90°. Viable sporangia were present in the early evening hours at the onset of high humidities, dew deposition, and temperatures below 75°. Assuming that they were discharged from the sporangiophores, these sporangia insure reinfection during the night. Hot, dry diurnal conditions, then, if followed by nocturnal air temperatures around 70° with dew deposition on the leaf surfaces, merely retard but do not kill the pathogen.

A most striking discovery was the fact that viable sporangia of the blight fungus were produced on potato foliage within 6 hours during the heat of the day. The viable sporangia produced during the afternoon provide a source of inoculum ready to develop at the onset of favorable nocturnal conditions.

[4] Crosier, W. 1934. Studies in the biology of Phytophthora infestans (Mont.) De Bary. N. Y. (Cornell) Agr. Exp. Sta. Mem. 155: 1-40.

[5] Melhus, I. E. 1915. Germination and infection with the fungus of the late blight of potato (Phytophthora infestans) Wis. Agr. Exp. Sta. Res. Bul. 37: 1-64.

SUMMARY

Blighted potato leaflets exhibiting no sporangia at 9 P.M. were covered with an abundance of sporangia at 8 A.M., 11 hours later.

Sporangia developed during the day on shaded and unshaded potato leaflets.

Viable sporangia were collected from blighted potato leaflets at 8 A.M., 9 A.M., 11 A.M., 2 P.M., 4 P.M., 8 P.M., 11 P.M., and 12 P.M.

Viable sporangia of *Phytophthora infestans* were found on potato and tomato foliage during afternoons when temperatures were in the 80's and during the following evening.

Sporangia collected from leaflets exposed to afternoon temperatures above 90° F. were not alive that evening.

During an afternoon of temperatures above 80° F. viable sporangia developed on potato leaflet lesions within 6 hours after they were brushed free of sporangia.

Viable sporangia were produced on potato leaflets during the 6 hours of temperatures above 80° F. and relative humidities in the 50's.

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STUDIES CONCERNING COLONIAL VARIANTS OF AGROBACTERIUM TUMEFACIENS [1]

C. Gainor and W. C. Price

Investigations on *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn, 1942, have dealt with smooth, rough, mucoid, and watery colonies (4, 9, 12, 14, 15, 20), and even the controversial filterable phase (3, 17). These studies concerned aspects of colony forms such as physiological behavior, spontaneous agglutination, serology, resistance to bacteriophages, and "microbic dissociation" (11), as this term was propounded by Hadley (7). However, the relationship of colonial variants of *A. tumefaciens* to phytopathogenicity has received scant attention, in marked contrast to comparable studies with animal pathogens (8).

This paper is concerned with the isolation of variants of *A. tumefaciens* differing in colonial morphology, their biochemical characterization, their phytopathogenicity, and their stability in culture and in plant tumors.

THE CULTURE.—The culture of *A. tumefaciens* used was that designated B6-highly virulent by A. C. Braun. A series of 6 nutrient agar (Difco) plates, pH 7.0, was serially streaked with the organism. After 72 hours' incubation at 30° C., there were approximately 400 well-separated, typically smooth colonies and 3 lobate ones on the plates, representing altogether 3 types of colony. A typical smooth B6-highly virulent colony was serially streaked onto 6 agar plates and incubated for 48 hours at 30° C. This procedure was repeated until 12 successive serial streakings at 48-hour intervals yielded 100 per cent smooth colonies, designated $B6_1$. In all instances of serial streakings the last 1 or 2 agar plates remained sterile.

ISOLATION OF VARIANTS.—Three flasks, each containing 100 ml. of nutrient broth (Difco) at pH 7, were each inoculated with a single $B6_1$ colony and incubated at 30° C. Serial streakings on nutrient agar plates were made daily for the first 7 days and then at weekly intervals. After 5 days' incubation in broth, the smooth form $B6_1$ gave rise to a mixed population of smooth colonies and lobate colonies with radial striations ($B6_2$). After approximately 14 days' incubation a third colony form ($B6_3$), presenting a more wrinkled appearance, grew on the streak plates. Whereas $B6_1$ could be suspended homogeneously in distilled water and physiological saline, the other 2 colony types were auto-flocculent. All 3 types presented a brownish growth on slices of potato tuber and were capable of growth on nutrient agar slants at 37° C. The 3 colony types isolated from a single stable smooth colony apparently are identical with the 3 observed in the original culture. Their changes in morphology with advancing age are depicted in Figure 1.

Simple staining techniques applied to 36-hour cultures, together with the Gram reaction, revealed cell structures similar to those reported by Smith et al (19, 20) and Rosen (17). Cells of $B6_3$ were sluggishly motile, whereas those of $B6_1$ and $B6_2$ were actively motile. No other differences among cells of the 3 types were observed.

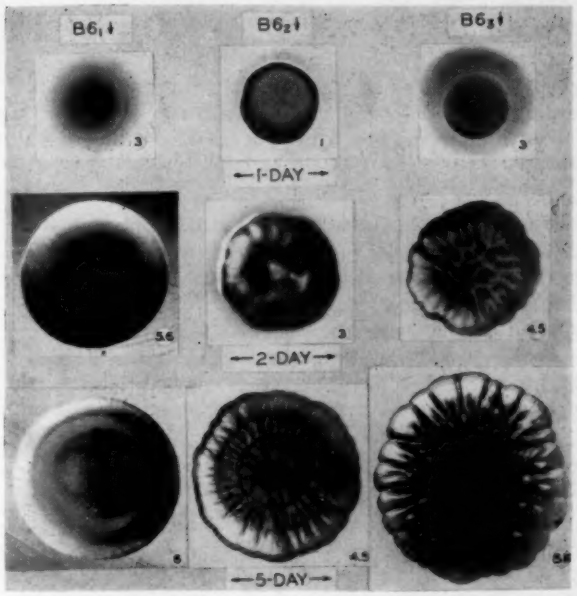

FIG. 1. Colonies of 3 types of *Agrobacterium tumefaciens* grown on nutrient agar at 30° C., and photographed after 1, 2, and 5 days. The numbers in the individual photographs refer to relative sizes of colonies when compared to 1-day-old $B6_2$ (1 = 0.7 mm. diameter).

A portion of the periphery from a 135-day-old $B6_1$ colony was suspended in sterile distilled water and subsequently streaked on a series of agar plates. The plates yielded opaque colonies ($B6_1A$, Fig. 2) which, when observed by transmitted light at 20× magnification after 3 days' incubation, appeared flat and granular with a slightly raised area approximately ¾ the diameter of the colony. The crimped periphery exhibited a narrow, translucent zone around the colony. The colonies were brittle but could be pushed around the surface of the agar without breaking. In appearance the colonies displayed characteristics intermediate between those of $B6_1$ and $B6_2$ (Fig. 1—5 days old). Similar isolation from the periphery of a 135-day-old $B6_2$ colony yielded colonies ($B6_2A$, Fig. 2) possessing a series of concentric ring formations when observed by transmitted light but otherwise identical with $B6_1A$. Peripheries of aged $B6_3$ colonies yielded opaque colonies ($B6_3A$, Fig. 2) with smooth surfaces and even borders, slightly butyrous in consistency, and having the general appearance of a 5-day-old $B6_1$ colony. These latter colonies went into suspension readily when dispersed in a tube of distilled water, whereas $B6_1A$ and $B6_2A$ colonies were auto-flocculent.

$B6_3$ was the only form subjected to preliminary observations in 0.5 per cent lithium chloride (7). Streak

[1] Accepted for publication May 26, 1953.

FIG. 2. Representative variants from $B6_1$, $B6_2$, and $B6_3$ after incubation at 30° C. on nutrient agar; $B6_3B$ (40×) after 5 days, others (20×) after 3 days. $B6_1A$, $B6_2$ A, and $B6_3$ A are the resulting colony types from periphery selections of 135-day-old colonies of $B6_1$, $B6_2$, and $B6_3$ respectively. $B6_3B$ and $B6_3C$ are isolates from a LiCl broth culture of $B6_3$.

plates made from each of the 25 serial subtransfers at 1- or 2-day intervals yielded 4 types of colonies: typical $B6_3$ forms; forms that appeared to fall between $B6_2$ and $B6_3$ types ($B6_3B$, Fig. 2); dull, opaque forms ($B6_3C$, Fig. 2) which, after 3 days' incubation, presented a curled periphery and friable central portion; and a mucoidal type. The latter reverted to a smooth opaque form (similar to $B6_1$) after 2 subtransfers on nutrient agar.

PATHOGENICITY STUDIES.—Cultures of $B6_1$, $B6_2$, and $B6_3$, 2, 4, and 8 days old, were suspended in sterile physiological saline and inoculated by needle puncture into 3 internodes of each of 3 plants of *Kalanchoe daigremontiana* Hamet & Perrier, 12 in. tall. Typical galls (6, 16) were produced at each inoculation site, appearing approximately 10 days after inoculation. No differences were detected in size, appearance, or position on the stem of the galls that could be correlated with colony type or age of the culture prior to inoculation. Representative colonies of the 3 types, upon reisolation from the galls, proved equally virulent on reinoculation into new *Kalanchoe* plants. $B6_1$, $B6_2$, and $B6_3$ were likewise pathogenic for plants of Holmes' necrotic type tobacco (a derivative of *Nicotiana digluta* Claussen & Goodspeed × *N. tabacum* L.), tomato (*Lycopersicon esculentium* Mill.), and Paris daisy (*Chrysanthemum frutescens* L.) and again no differences among the galls produced by the 3 cultures were detected. All isolates depicted in Figure 2 were pathogenic for *Kalanchoe*.

STABILITY OF COLONY FORMS IN GALLS.—Galls of varying ages, 30, 50, and 72 days old, were aseptically cut from the stems of the inoculated plants, immersed in individual vials of saturated calcium hypochlorite solution for 5 seconds, neutralized in a saturated solution of sodium thiosulfate, and triturated in 1 ml. sterile distilled water with sterile mortar and pestle. Serial streak plates were made from the ground-gall suspension. Three galls of each colony type were sampled at each interval studied.

Galls removed 30, 50, and 72 days after inoculation indicated almost 100 per cent presence of organisms in the original colony form. The only exceptions were a $B6_1$ gall (30-day sampling) which yielded cultures containing approximately 2 per cent of the $B6_3$ type, and a $B6_3$ gall (72-day sampling) which yielded a few organisms of the $B6_1$ type. In both instances only 1 of the 3 galls removed at time of sampling yielded the additional types. The surprising absence of contaminants on streak plates made from the gall suspension attests to the efficacy of the hypochlorite-thiosulfate treatment for surface sterilization of removed galls. Random selecions of representative colony types reisolated from the galls gave characteristic morphological, biochemical, and pathogenic reactions.

STABILITY OF COLONY FORM IN CULTURE.—Although no attempt was made to evaluate quantitatively the transformation from one colonial type to another, it was noted that after 5 days' incubation in nutrient broth the $B6_1$ culture began to transform to the $B6_2$ type. After 14 days' incubation the $B6_3$ variety made its appearance in limited numbers (in contrast to the preponderance of $B6_1$ and $B6_2$ forms). After 35 days the $B6_2$ was present in approximately equal numbers with the $B6_1$ type. The $B6_2$ form was stable throughout a 35-days incubation period, whereas the $B6_3$ colonies demonstrated a shift toward $B6_1$ after 14 days' incubation, with an occasional colony of the $B6_2$ variety. After 35 days a mucoidal type made its appearance but altered to a smooth form after 2 subtransfers on nutrient agar. At frequent intervals Gram stains were made from the broth flasks to determine possible presence of contaminants. None were found. Examination of the cultures after 140 days' incubation indicated that they were still viable.

The stability of the 3 colony types was maintained for more than 9 months under conditions of subtransfer of individual colonies from nutrient agar streak plates to other agar plates at 2- to 4-day intervals. The same degree of stability was maintained on agar slants without subtransfer after 3 months' incubation at 5° C. This greater stability on agar than in nutrient broth has been noted for other phytopathogenic bacteria (13).

$B6_1A$, $B6_2A$, $B6_3A$, $B6_3B$, and $B6_3C$, after initial isolation, remained stable for 3 successive transfers on nutrient agar. However, after remaining on agar slants for 3 months at 5° C., only $B6_3A$ and $B6_3B$ displayed constancy of colony form. $B6_1A$, $B6_2A$ and $B6_3C$ reverted to the smooth $B6_1$ colony type.

BIOCHEMICAL TESTS.—To 4.5 ml. of sterile nutrient broth was added 0.5 ml. of a Seitz-filtered solution of chemically pure sugars or alcohols, so that the final concentration of the carbohydrates was 1 per cent. All tubes were at pH 7.1 prior to inoculation with organisms and each contained an inverted Durham fermen-

tation tube for detection of gas. Because of limited solubilities, saturated solutions of inulin, dextrin, salicin, and dulcitol were employed. Each reaction tube was inoculated with 0.1 ml. of a suspension of the colony form under consideration. The inocula were prepared by selecting typical colonies grown on agar plates for 48 hours and suspending the organisms in sterile distilled water to the desired turbidity of approximately 200 million bacteria per ml. In addition to tests for indole, acetyl-methylcarbinol, citrate utilization, nitrate reduction, gelatin liquefaction, and reaction in skim and litmus milk, the following carbohydrates were checked for fermentation: arabinose, cellobiose, dextrin, dextrose, dulcitol, galactose, glycerol, inositol, inulin, lactose, levulose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. Acidity in the carbohydrates was determined by change in reaction with Andrade's indicator and by a Beckman pH meter. The other tests were conducted according to standard procedures (5). All tests were done in quadruplicate, and the tubes were incubated at 30° C.

Since the purpose of the biochemical studies described above was to ascertain whether differences in behavior were correlated with colony form, readings were taken after 4 days' incubation. Studies conducted in nutrient broth as well as in carbohydrate broths indicated that the organisms had not undergone transformation during this interval.

Of the 20 carbohydrates tested, arabinose, cellobiose, dextrose, galactose, levulose, and xylose were fermented to acid without gas by $B6_1$, $B6_2$, and $B6_3$. $B6_2$ was generally the most active colony type as measured by H-ion concentrations. The ranges of pH results were 5.7 ($B6_2$) to 6.4 ($B6_1$ and $B6_3$) for arabinose and xylose, whereas the other 4 fermented sugars gave readings between pH 6.4–6.9. Levulose gave the weakest response (pH 6.7–6.9). $B6_1$A, $B6_2$A, $B6_3$A, $B6_3$B, and $B6_3$C gave the same general results as noted above. Without carbohydrates, the final pH readings were approximately 8.0.

Results of the other biochemical tests are in general agreement with those recorded in Bergey's manual (2).

In litmus milk and sterile skim milk, $B6_1$ and $B6_3$ formed, within 2–4 days, a serum zone and a residuum of fine flocculent material of casein (10). $B6_2$, however, did not effect the same reaction until after 12–14 days' incubation. Streak plates made from the $B6_2$ tubes having a serum zone indicated 2 types of colonies: typical $B6_2$ colonies, and flatter colonies with a granular surface and without lobate development. The latter colonies produced a serum zone in freshly inoculated milk after 3 days' incubation, whereas the typical $B6_2$ colonies once again failed to produce a serum zone. This instability of $B6_2$ in milk is in marked contrast to its stability for 35 days in nutrient broth. The reactions in milk with all 3 colony types were either slightly alkaline or neutral. These findings are in agreement with those of Israilsky (11) and Hendrickson, Baldwin, and Riker (9). Wormald (22) noted different reactions in milk with different strains of *A. tumefaciens*. Serum zones were produced by $B6_1$A, $B6_2$A, $B6_3$A, $B6_3$B, and $B6_3$C.

Tests for nitrate reduction were carried out in nitrate broth (Difco) and on nitrate agar (Difco) slants. After 4 days' incubation all broth tubes gave a positive test for ammonia production, whereas all the agar slants gave sharp positive nitrite reactions.

Tests for indole, acetylmethylcarbinol, and gelatin liquefaction were negative.

Discussion.—Surprisingly few photographs of the colonies of *A. tumefaciens* are available in the literature for comparative purposes. Our $B6_1$ and $B6_2$ colonies, 5 days old, are similar to those illustrated by Israilsky (10) in "Abb. 3" (left picture) and "Abb. 4," respectively. Our $B6_3$ is comparable to "Abb. 6" in his subsequent publication (11) in which he mentions that any of his colony types of *A. tumefaciens* can assume all the forms depicted in his photographs. Colonies in the series of photographs in the paper by Link and Hull (12) are not similar to ours, with the exception of their typical smooth colonies which are more like our 2-day-old $B6_2$.

The ability of a number of phytopathogenic species to change from smooth to rough has been noted (18, 21). With the exceptions of $B6_1$ (Fig. 1) and $B6_3$A (Fig. 2), most of the colonies depicted in this paper could be designated "rough" according to common usage in the literature concerning *A. tumefaciens*. The usual characteristics attributed to rough colonies of human and animal pathogens (7, 8), such as translucency, flatness with uneven peripheries, and filamentous cell structures, are not always applicable to the recorded examples of *A. tumefaciens*.

Valleau, Johnson, and Diachun (21) have found, in nature, strains of *Pseudomonas tabaci* (Wolf & Foster) Stevens and *P. angulata* (Fromme & Murray) Holland ranging from smooth to watery to all degrees of roughness in colonial characteristics. The authors indicate that these various forms are relatively stable and do not differ in ability to infect their hosts. A study on *Xanthomonas phaseoli var. sojense* (Hedges) Starr and Burkholder (18) showed that the smooth phase of this organism was stable after inoculation into soybean plants, whereas the rough strains gave rise to both rough and smooth types of colonies. Whereas most workers agree that both the smooth and wrinkled colony forms of *A. tumefaciens* are generally equally virulent, work with other plant pathogens has shown differences in virulence correlated with differences in colony form. Sharp (18) reported that his smooth colony form of *X. phaseoli* var. *sojense* was more virulent than his rough colony type in causing pustules on soybean. Quirk (15) reported isolation of a rough form of *A. tumefaciens* that was avirulent but gave no detailed description of the morphology of the rough isolate nor its stability. Our studies with this organism have indicated remarkable stability of colony form of the 3 primary types ($B6_1$, $B6_2$, and $B6_3$) in galls on *Kalanchoe*. Persistance in galls on other plant

species (16) and in soil (1) has been noted previously.

SUMMARY

A study in bacterial variation with a strain of *Agrobacterium tumefaciens* yielded 3 forms ($B6_1$, $B6_2$, and $B6_3$) differing in colonial morphology. The interrelationship between the colonial forms was established.

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PHYSIOLOGIC DIFFERENCES BETWEEN TWO PHYSIOLOGIC RACES OF PHYTOPHTHORA INFESTANS [1]

Alex M. French [2]

The importance of physiologic races of the late blight pathogen, *Phytophthora infestans* (Mont.) DeBary, in breeding potatoes resistant to the disease has been emphasized in a recent review article by Stevenson, Schultz, Akeley, and Cash[3]. With the discovery of apparent immunity in the wild potato species *Solanum demissum* Lind., and the production of "blight-immune" hybrids between the wild form and *S. tuberosum* L., breeding the ideal blight-immune commercial varieties appeared to be only a matter of time. Unfortunately, while varieties showing greater degrees of resistance to the common field race of *P. infestans* were being developed in this country and abroad, there appeared more virulent races of the fungus which could infect even the most resistant potato varieties. Such races developed naturally in the field, and could also be produced under controlled conditions in the greenhouse.

An understanding of the physiologic basis of the plasticity of *Phytophthora infestans* should furnish a clue to the nature of blight resistance in the potato. One approach to this problem, that of comparing *in vitro* the nutritional responses of the field race with those of one of the more virulent races, has been attempted by the writer.

MATERIALS AND METHODS.—The 2 cultures of *Phytophthora infestans* employed were obtained from Dr. L. C. Peterson of the Department of Plant Pathology at Cornell University, Ithaca, New York. The origin of the isolates was as follows:

Field Race A (Isolate C-14): isolated in 1946 from naturally-infected potatoes of the variety Green Mountain.

Race B (Isolate AAB-2): developed by Dr. W. R. Mills from Field Race A by passage through the potato varieties President, KB-5, and AAB-2. The varieties named represent a series with increasing degrees of resistance to late blight.

Pathogenicity of these cultures was checked at intervals during the course of the studies by inoculating leaves of test plants of Green Mountain and AAB-2 potatoes. Isolate C-14 consistently infected only the susceptible Green Mountain variety, while Isolate AAB-2 infected both Green Mountain and the highly resistant variety AAB-2.

Stock cultures were maintained on slants of lima bean agar prepared by mixing 200 gm. of frozen Fordhook lima beans with 300 ml. distilled water in a Waring Blendor, and adding this mixture to 500 ml. of hot distilled water in which 15 gm. of agar had been dissolved. To maintain the cultures in a vigorous condition of growth and sporulation, new transfers were made to freshly-prepared slants at intervals of 2 weeks or less.

To obtain large quantities of swarmspore suspensions for seeding the liquid culture flasks, transfers were made about 2 weeks in advance to lima bean agar in Roux culture bottles, which provided large surfaces for sporulation. When inoculum was desired, a sufficient quantity of sterile glass-distilled water was added aseptically to each culture bottle, shaken over the agar surface to dislodge the sporangia, and held in the bottle for 3 hours at 12° C. Swarmspore counts were then made, using a Levy counting chamber under the low power of the microscope, and additional cool sterile distilled water was added if necessary to make the swarmspore concentrations roughly comparable. The suspensions usually contained approximately 400,000 swarmspores per ml. One ml. of swarmspore suspension was transferred from a culture bottle to each culture flask by means of a sterile pipette.

Quadruplicate 150-ml. Erlenmeyer flasks containing 20 ml. of nutrient solution were employed for all the liquid culture studies. Glass-distilled water was used throughout the experiments, and all glassware was cleaned with chromic acid cleaning mixture and rinsed with glass-distilled water. The pH of each culture solution was adjusted to 5.50 before autoclaving at 15 lbs. pressure for 20 minutes. The flasks were cooled to approximately 21° C. before seeding, and the cultures were incubated at 21° C. for approximately 3 weeks.

Growth was measured by filtering, drying, and weighing the mycelium from each flask individually in a matted and tared Gooch crucible. The final pH of each solution was determined with the Beckman glass-electrode pH meter.

EXPERIMENTAL RESULTS.—*Nitrogen sources.*—In preliminary experiments, differences were detected between Field Race A and Race B in their utilization of different nitrogen sources for growth. A factorial experiment was devised, offering all possible combinations of 4 nitrogen sources, 2 organic and 2 inorganic, for the growth of the 2 races.

The base medium, which will hereafter be referred to as Solution A, had the following composition, in gm./1: KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.25; dextrose, 25.0; and thiamin, 1 part per million. The nitrogen sources employed were KNO_3, $(NH_4)_2SO_4$, asparagine, and Bacto-peptone. Each was used at a con-

[1] Accepted for publication May 23, 1953.
Based on a thesis submitted to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author gratefully acknowledges the assistance of Drs. G. C. Kent, L. C. Peterson, A. F. Ross, and A. W. Dimock throughout the course of the investigations and in the preparation of the manuscript.

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[3] Stevenson, F. J., E. S. Schultz, R. V. Akeley, and L. C. Cash. 1952. Late blight immunity in the potato. Phytopathology 42: 277-280.

TABLE 1.—*Mean dry weight of mycelium of Races A and B of Phytophthora infestans grown in Solution A[a] with various combinations of nitrogen sources*

N Sources[b]	Total N per flask (mg)	Race A Dry wt. (mg)	Race B Dry wt. (mg)
None	0.0	1.3	1.2
Am ($(NH_4)_2SO_4$)	4.2	1.3	3.9
Ni (KNO_3)	2.8	1.8	6.4
Am-ni	7.0	2.5	4.9
As (Asparagine)	4.2	2.5	42.1
Am-As	8.5	1.0	35.3
Ni-As	7.0	2.4	49.0
Am-Ni-As	11.3	2.0	34.7
Pt (Peptone)	3.2	23.3	38.8
Am-Pt	7.5	4.2	21.4
Ni-Pt	6.0	30.7	51.0
Am-Ni-Pt	10.2	4.8	24.1
As-Pt	7.5	46.2	58.8
Am-As-Pt	11.7	2.0	28.5
Ni-As-Pt	10.2	2.3	56.6
Am-Ni-As-Pt	16.5	0.7	26.2
LD 99:1		3.7	12.7

[a] The base medium included the following, in gm./1: KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 0.25; dextrose, 25.0; thiamin, 1 ppm.
[b] Each nitrogen source was added at a concentration of 1.0 gm./1.

centration of 1.0 gm./1.

The results of this experiment (Table 1) indicated that:

1) Bacto-peptone was an excellent source of nitrogen for both races;

2) KNO_3, at a concentration of 1.0 gm./1, was not a good source of nitrogen for either race;

3) Asparagine was as favorable a source of nitrogen as was peptone for Race B, while it was not readily utilized by Field Race A;

4) $(NH_4)_2SO_4$ was not a good source of nitrogen for either race; in fact, at the concentration of 1.0 gm./1, it appeared to be distinctly inhibitory to both races;

5) There was no correlation between growth of either race and total nitrogen content of the various solutions.

To verify the relative value of peptone and asparagine as nitrogen sources for the 2 races, and to test other organic sources of nitrogen, a further experiment was performed in which the total quantity of elemental nitrogen was the same in all the solutions. The nitrogen sources used, and the quantities added per liter of Solution A to make each culture flask contain the equivalent of 10 mg. N, were as follows:

1—Asparagine	2.36 gm.
2—Bacto-peptone	3.09 gm.
3—Asparagine	1.34 gm.
Bacto-peptone	1.34 gm.
4—l-Aspartic acid	4.75 gm.
5—l-Aspartic acid	0.78 gm.
d-Glutamic acid	0.78 gm.
d-Arginine	0.78 gm.
Glycine	0.39 gm.
dl-α-Alanine	0.39 gm.
6—No nitrogen	

TABLE 2.—*Mean dry weight of mycelium of Races A and B of Phytophthora infestans grown in Solution A[a] with various organic nitrogen sources*

Nitrogen Source[b]	Race A Dry wt. (mg)	Race B Dry wt. (mg)
Asparagine	0.6	58.9
Bacto-peptone	44.5	59.1
Asparagine, Bacto-peptone	29.4	61.0
Aspartic Acid	0.3	31.5
Amino acid mixture (Aspartic acid, glutamic acid, arginine, glycine, alanine)	10.6	55.4
None	1.0	2.4
LD 99:1	6.0	9.1

[a] The base medium included the following, in gm./1: KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 0.25; dextrose, 25.0; thiamin, 1 ppm.
[b] The total concentration of elemental nitrogen in each of the solutions was equivalent to 10 mg. per culture flask.

The mixture of amino acids (Solution 5) was suggested by the work of Leonian and Lilly.[4]

Results of this experiment (Table 2) indicated that Field Race A was unable to utilize certain amino acids as readily as more elaborated nitrogen compounds, while Race B was more versatile as to its utilization of organic forms of nitrogen. In particular, it should be noted that the Field Race failed to utilize asparagine or aspartic acid.

The apparent inhibition of growth by the presence of $(NH_4)_2SO_4$ in the nutrient solution was investigated further in an experiment in which different quantities of $(NH_4)_2SO_4$ were added to the medium. Six solutions were prepared, using as a base medium Solution B, which contained, in addition to the ingredients of Solution A, 1.0 gm./1 asparagine and 1.0 gm./1 KNO_3. $(NH_4)_2SO_4$ was introduced in concentrations of 0.0, 0.1, 0.2, 0.5, 1.0, and 2.0 gm./1.

The growth of both Field Race A and Race B was reduced by the addition of $(NH_4)_2SO_4$, but at different

[4] Leonian, Leon H., and Virgil Greene Lilly. 1940. Studies on the nutrition of fungi. IV. Factors influencing the growth of some thiamin-requiring fungi. Amer. Jour. Bot. 27: 18-26.

TABLE 3.—*Mean dry weight of mycelium of Races A and B of Phytophthora infestans grown in Solution B[a] with different concentrations of ammonium sulfate added*

$(NH_4)_2SO_4$ Concentration gm./l.	Race A Dry wt. (mg)	Race B Dry wt. (mg)
0.0	20.5	59.3
0.1	20.8	51.6
0.2	24.5	49.6
0.5	5.3	48.5
1.0	4.0	50.2
2.0	0.4	30.1
LD 99:1	15.2	4.2

[a] The base medium contained the following, in gm./1: KH_2PO_4, 0.5; KNO_3, 1.0; $MgSO_4 \cdot 7H_2O$, 0.25; asparagine, 1.0; dextrose, 25.0; thiamin, 1 ppm.

TABLE 4.—*Mean dry weight of mycelium of Races A and B of Phytophthora infestans grown in Solution B[a] with succinic acid and/or ammonium nitrate added*

Culture Solution	Race A Dry wt. (mg)	Race B Dry wt. (mg)
Soln. B	22.2	50.3
Soln. B + Succinic acid[b]	2.7	50.4
Soln. B + NH_4NO_3	6.8	36.3
Soln. B + Both	1.3	60.8
LD 99:1	5.9	7.3

[a] Composition of Solution B is given in footnote, Table 3.
[b] Succinic acid and NH_4NO_3 were added at the rate of 1.0 gm./1.

concentrations (Table 3). Inhibition of growth of Field Race A took place between 0.2 and 0.5 gm./1, while for Race B the greatest reduction occurred at a concentration of 2.0 gm./1. A repetition of this experiment verified these results.

In the nutrition of certain fungi, including *Phytophthora erythroseptica*, the addition of succinic acid to the medium has been reported to enhance the utilization of NH_4NO_3 and other nitrogen sources.[4] This relationship was tested by an experiment in which succinic acid and NH_4NO_3 were added together and separately to Solution B, at concentrations of 1.0 gm./1.

Ammonium nitrate inhibited the growth of both races to about the same extent as did ammonium sulfate (Table 4). Succinic acid had a distinctly adverse effect on the growth of Field Race A, either alone or in combination with NH_4NO_3. For Race B, on the other hand, succinic acid had no significant effect on growth, except for an apparent synergism in combination with NH_4NO_3.

Differential agar medium.—Using the results of the foregoing liquid culture studies as a guide, a solid medium was prepared which acted as a differential for the 2 races of *Phytophthora infestans*. The composition of this medium, in gm./1, was KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.25; KNO_3, 1.0; asparagine, 1.0; NH_4NO_3, 1.0; succinic acid, 1.0; dextrose, 25.0; agar, 15.0; and thiamin, 1 p.p.m.

The differential nature of this medium was striking when transfers of Field Race A and Race B cultures were made onto poured petri dishes. Field Race A made no diametric growth from the transfer block, while Race B regularly covered most of the surface of the agar within 2 weeks' incubation at 21° C.

Supplementary growth factors.—Throughout the experiments dealing with nitrogen sources, it was noted that the growth of Field Race A was considerably poorer than that of Race B. An attempt was made to induce more luxuriant growth of the Field Race by adding materials which might contain supplementary growth factors. In an exploratory experiment, a 20-fold increase in growth was observed when a commercial raw sugar preparation[5] was used in place of dextrose, and a 10-fold increase when Bacto-yeast extract was added at a concentration of 5.0 gm./1.

Characterization of the growth-promoting substance present in raw sugar and yeast extract was attempted through fractionation of each material by alcohol and ether extraction, dialysis, ashing, and adsorption by and elution from fuller's earth. Growth of Field Race A and Race B was tested in media containing Solution B plus quantities of each fraction equivalent to 25 gm./1 of raw sugar or 5 gm./1 of yeast extract. In the sugar series, a concentration equal to 25 gm./1 of sucrose was attained in each solution by testing with a refractometer and adding an appropriate quantity of C.P. sucrose.

The remarkable stimulation in growth of Field Race A was again observed in this experiment, both with raw sugar and yeast extract, and with several of the fractions of each. Race A appeared to respond to a growth factor present in both raw sugar and yeast extract which was non-dialysable, insoluble in ether, partially soluble in absolute alcohol, not adsorbed by fuller's earth in acid solution, and not present in the ash.

Growth of Race B was not stimulated by addition of raw sugar. A highly significant stimulation in growth of Race B did occur, however, through addition of yeast extract, and the growth stimulant appeared to be dialysable, insoluble in ether or absolute alcohol, and not adsorbed by fuller's earth.

A number of vitamins, including biotin, riboflavin, nicotinic acid, pyridoxine, inositol, pantothenic acid, choline, p-aminobenzoic acid, and β-alanine, as well as a preparation of sodium nucleate from yeast, were tested as possible growth factors by adding them individually to Solution B. All the vitamins failed to stimulate growth of either race at the concentrations tested, but a highly significant increase in growth of Field Race A was noted when yeast nucleic acid salts were present in a concentration of only 10 parts per million. The same material had no effect on growth of Race B.

DISCUSSION.—Inhibition of growth by the presence of ammonium ions in the substrate has not previously been reported for *Phytophthora infestans*, although it has been noted in similar studies with the closely-related species *P. phaseoli*.[6] A possible physiological explanation of the ammonium ion inhibition is suggested by the work of Knight,[7] who found that the l-amino acid oxidase of *Penicillium chrysogenum* was inhibited by addition of ammonium sulfate to its substrate.

Although it was impossible to complete the identification of the growth factors which appeared to be present in raw sugar and yeast extract, it was shown that they are not among the common vitamins, and

[5] "Grans" Free-flowing Raw Sugar Granules, National Sugar Refining Company.

[6] Saksena, R. K., and K. S. Bhargava. 1943. Nitrogen requirements and vitamin deficiencies of *Phytophthora phaseoli* Thaxter. Indian Acad. Sci., Proc. 18: 45-51.

[7] Knight, S. G. 1948. The l-amino acid oxidase of molds. Jour. Bact. 55: 401-408.

that the factors which stimulate the growth of Field Race A and Race B are apparently not the same.

Inclusion of the sodium nucleate preparation in testing for growth-promoting activity was prompted by the observation that the factor in yeast extract which stimulated growth of Field Race A was non-dialysable. The stimulation that occurred when the nucleic acid salt was added suggests that this growth factor may be found among the purine or pyrimidine bases, nucleotides, or nucleosides.

SUMMARY

Differences in nutritional responses of the common Field Race A of *Phytophthora infestans* and Race B, which is pathogenic to various blight-resistant potato hybrids, have been discovered.

Asparagine and certain amino acids were poor nitrogen sources for Field Race A in synthetic liquid media, whereas Race B utilized them effectively.

Ammonium salts and succinic acid were inhibitory to Field Race A, but failed to inhibit growth of Race B at the same concentrations.

A component of raw sugar acted as a powerful growth stimulant for Field Race A, but left Race B unaffected. Yeast extract was found to contain a non-dialysable growth factor for Race A and a dialysable factor for Race B.

A synthetic agar medium was formulated which supported the growth of Race B but not of Field Race A.

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COMPARISONS OF HELMINTHOSPORIUM SPECIES ATTACKING OATS IN FLORIDA [1]

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This paper deals with *Helminthosporium victoriae* Meehan & Murphy (10), *H. sativum* Pam., King & Bakke (12), and a *Helminthosporium* closely approximating *H. sativum* isolated from Southland Oats (C.I. 5207). These fungi cause foot- and culm-rots, spot blotches and blights of cereals and grasses and, according to Luttrell's key (8), are morphologically closely related. Nisikado (11) places all *Helminthosporium* of this type in his sub-genus *Eu-Helminthosporium*, characterized as having long elliptical, obclavate or obovate fusiform, typically curved conidia, more or less tapered toward both ends and germinating from either polar cell.

Since the description of *H. victoriae* in 1946 (10) there have been some differences of opinion as to its validity as a species because of its marked similarity to *H. sativum*. This similarity became more apparent when isolations from culm tissues of Southland Oats (5) gave fungi morphologically similar to *H. sativum*, but which damaged the crowns and lower sections of the culms of oats as does *H. victoriae*. While the correct taxonomic designation of this species has not been determined, the disease it incites on oats in Florida has been tentatively called "culm rot."

When these morphological and physiological similarities became apparent, it was thought advisable to compare the pathogenic behavior of these fungi on some commonly-grown small grains of this region. This paper reports the results of inoculation experiments with these 3 fungi on different small grain crops and a comparison of their conidial sizes.

Methods and materials.—An isolate of *Helminthosporium victoriae* (H-96), supplied by H. C. Murphy, was compared with isolates of *H. sativum* from wheat and barley and an isolate (G-3) of the fungus causing culm rot of Southland oats. The fungi were grown for 3 weeks on 20 cc. slants of standard potato-dextrose agar in 6 oz. medicine bottles. Aqueous suspensions were prepared for inoculations by macerating the entire contents of the bottles with 150 cc. of tap water in a Waring blendor for 2 minutes. The fungal suspensions were atomized on the foliage of 3-week-old seedlings of C.I. 3852 barley[3], Victorgrain and Southland oats, Florida Black rye, and Atlas 66 wheat.

After inoculations, the test plants were held at approximately 100 per cent relative humidity for 48 hours. Diurnal temperature fluctuations were from 68° at night to 83° during the day. Humidity control was not exact since this factor was controlled only by the evaporation of water from soaked sphagnum moss in a closed chamber. This chamber was constructed of a transparent plastic screen to prevent etiolation of the seedlings. Upon removal from the moist chamber the test plants were put on greenhouse benches where they were subjected to the same temperature changes.

Infection ratings were secured 48 hours after removal from the moist chambers. These infection ratings were made with "0" indicating no infection and "4" indicating a severely damaged plant that was either dead or was incapable of surviving. The latter 2 categories were given the same infection rating

[1] Accepted for publication June 14, 1953.
Contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Dept. of Agriculture, and Plant Pathology Dept., Florida Agricultural Experiment Stations, Gainesville. Florida Agricultural Experiment Station Journal Series, No. 157.

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[3] C.I. refers to accession number of the Division of Cereal Crops and Diseases, U. S. D. A.

TABLE 1.—*Comparison of the infectivity of 4 isolates of Helminthosporium when inoculated on the foliage of 4 different grain crops*

1950 Test

Isolate	Index of infection on[a] Fla. Black Rye	Atlas 66 Wheat	C.I. 3582 Barley	Victorgrain Oat	Southland Oat
Helminthosporium sp. (G-3)	0.2	1.8	3.5	2.2	4.0
H. sativum (barley)	0.1	2.9	4.0	0.1	1.3
H. sativum (wheat)	2.7	3.8	3.8	0.0	0.3
H. victoriae (H-96)	0.0	0.0	0.3	4.0	0.2

1952 Test

Isolate	Fla. Black Rye	Atlas 66 Wheat	Suwan #6 Barley	Victorgrain Oat	Southland Oat
Helminthosporium sp. (H-37)	0.1	2.2	3.2	2.4	4.0
H. sativum (barley)	0.1	3.1	4.0	0.0	1.0
H. sativum (wheat)	3.2	4.0	3.9	0.0	0.4
H. victoriae (H-96)	0.0	0.0	0.1	4.0	0.2

[a] Infection indices are based upon a 0-4 Scale with "0", no infection, "4", severely damaged. Average response of 100 plants.

since experience had indicated that plants severely damaged, but not dead at the end of 48 hours would eventually die, usually within 72 hours after removal from the moist chamber. A longer period of time was not allowed before rating infections because the plants in the more resistant categories were the easiest to separate at the end of 48 hours.

In the second series of inoculations Suwan #6 barley was substituted for C.I. 3582, for which additional seed was not available, and isolate (H-37) of the *Helminthosporium* sp., isolated from oat culm tissue, was substituted for culture (G-3). Culture (H-37) was a single spore isolate from the mass culture (G-3) obtained from Southland Oats.

RESULTS.—The infection ratings are in Table 1.

In these series of inoculation experiments it was demonstrated that pathogenic behavior of any individual isolate is peculiar to itself, with no 2 provoking identical responses from this group of test plants. Although there are certain isolates that tend to behave alike, i.e., the *H. sativum* isolated from barley and the one from wheat, where the correlation coefficient "r" of their infection responses was 0.67; and the isolate from barley with the *Helminthosporium* sp. isolate from Southland which gave an "r" value of 0.48. In this test an "r" value of 0.43 was necessary for significance at the 5 per cent level. Whereas, the response of the isolate from Southland and *H. victoriae* gave an "r" value of —0.59 which indicates that these behaved differently.

DISCUSSION.—The analyses of these data indicate that identical responses were not obtained for any 2 of these fungi on this particular set of test plants, although some were similar. Calculations of correlation coefficients indicate that the isolates from barley, wheat, and the isolate from Southland Oats are 1 species, *Helminthosporium sativum*, the difference in response probably being due to physiological specialization. While adequate work is not yet available for establishing races of this species, as has been done with other cereal pathogens; Christensen (2) and Luttrell (9) have given evidence to indicate that physiologically identifiable groups exist.

We are considering the fungus isolated from Southland Oats as being *H. sativum*. This was indicated by F. Meehan, J. J. Christensen, and J. H. Miller, who identified the fungus in 1950. These mycologists were all in agreement that these isolates morphologically most nearly fit the description of *H. sativum*. However, at that time, a search of the literature revealed no data indicating that this fungus was pathogenic on oats, although several reports existed of its isolation from oats. Since then, J. J. Christensen has advised the author that this phenomenon had been observed, although published information as to its occurrence is rare. It was felt that since this fungus from Southland attacked oats in about the same manner as does *H. victoriae*, that it would be more nearly similar in behavior to *H. victoriae* than to fungi attacking wheat and barley. The data in Table 1 indicated that this was not the case; their pathogenic behavior was entirely different.

It has been demonstrated by Drechsler (4), Christensen (1,2,3) and Elliot (6) that extreme intra-specific morphological variability exists in this group of fungi. This variability within *H. victoriae* and *H. sativum* is frequently greater than the morphological differences that separate them as species. As an example of this latter phenomenon some conidial measurements and numbers of septations for these 2 species have been recorded in Table 2. It is realized that the characters cited in this table probably represent the maximum in morphological variability; nevertheless, they are important characters in distinguishing these species.

TABLE 2.—*Conidial measurements approximated to nearest whole micron and numbers of conidial septations in Helminthosporium sativum and Helminthosporium victoriae*

Length		Max. Width	No. of Septa	Source
Helminthosporium sativum				
a. from oats				
40-85	×	16-22	3-10	Lowery (7)
39-94 (62)[a]	×	15-22 (19)	3- 8 (6)	[b]
b. from barley				
40- 90	×	16-22	3-10	Lowery (7)
60-120	×	15-20	3-10	Sprague (13)
105-130	×	15-20	7-14	Pammel (12)
38- 90 (64)	×	16-21 (18)	3-99 (6)	[b]
Helminthosporium victoriae				
a. from oats				
40-130 (70)	×	11-25 (15)	4-11 (8)	Meehan (10)
32-115	×	9-19	2-10	Lowery (7)
36-122 (56)	×	10-28 (18)	2-10 (7)	[b]

[a] Numbers in parenthesis are modal measurements.
[b] Fungi used in these experiments: *H. sativum* (oats) isolate (H-37), *H. sativum* from barley same as in tests, *H. victoriae* (H-96) on Victorgrain oats.

Elliot (6) demonstrated that slight changes in environmental conditions may result in wide differences in the morphology of these fungi. Certain important morphological characters of these fungi, such as those given above, overlap in various ways so that they prevent their use for making clear-cut specific determinations. Therefore, other morphological characters or physiological responses must be used to separate them. Before this can be accomplished a large amount of data from inoculation experiments needs to be collected and processed. However, these organisms are of sufficient economic important that the establishment of exact methods of differentiating them should be undertaken.

SUMMARY AND CONCLUSIONS

Inoculation experiments were made on 5 different small grain crop varieties with 4 different Helminthosporium cultures: *H. victoriae* (H-96), *H. sativum* isolates from wheat and barley, and an isolate from Southland Oats similar to *H. sativum*. Differential host reactions were recorded and a compilation of conidial measurements presented.

From this study there appears adequate evidence for maintaining 2 species, *Helminthosporium sativum* and *H. victoriae*. However, it is suggested that a thorough and systematic study would result in a more exact classification of these species based upon morphological characters supplemented by physiological behavior.

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THE SCLEROTINIA DISEASE OF BEANS IN IDAHO[1]

C. W. Hungerford and Ralph Pitts[2]

The white mold disease on beans caused by *Sclerotinia sclerotiorum* (Lib.) deBary is probably world wide in its distribution. Harter and Zaumeyer (6) indicate a wide distribution in foreign countries and state that it may be found some seasons in bean fields in practically every part of the United States where weather conditions are favorable for its development.

The host range for *S. sclerotiorum* is wide. Anderson (1) and Young (9) have given rather complete lists of plants susceptible to this fungus. Tanrikut and Vaughan (8) investigated the nutrient requirements of *S. sclerotiorum* and found that it has the ability to grow in almost any substrate and thus can live for long periods as a saprophyte. They concluded that this extreme adaptability and its habit of producing tough, resistant sclerotia make it a very difficult fungus to control.

Occurrence in Idaho.—The white mold disease has been observed in Idaho on beans and other crops for many years but was not of much economic importance on beans until recently. Blodgett (3) gave an excellent discussion of the disease and found evidence indicating that by 1940 it had become a significant factor in bean production. Since that time, it has greatly increased in importance until in some localities, when environmental conditions are favorable, it is a serious disease of field and garden beans.

The incidence and importance of *S. sclerotiorum* on beans in Idaho was investigated by KenKnight and Blodgett (7) in 1943. They found the disease more generally distributed and responsible for greater damage to the bean crop than they had expected. The survey was continued by Blodgett during 1943–1944 and 1944–1945.

Blodgett (3) concluded that the prevalence of sclerotia in samples from cleanings and in cull piles was a reasonably accurate indication of field conditions as regards infection and loss.

A question has arisen concerning this method of sampling. Are these sclerotia in the cull piles a true index of field damage or do they represent partially spread of the fungus occurring after the crop has been cut and waiting to be threshed? It seems possible that a bean crop might be infested with white mold to a minor degree before it is cut and piled in windrows; if the temperature and moisture are favorable the fungus could spread rapidly among the piled bean plants forming numerous sclerotia. It would give an exaggerated idea of the damage done to the bean crop provided the number of sclerotia in the cull bean piles is used as an index of the loss caused by white mold to the growing bean crop.

Commercial companies after cleaning beans often dump the cleanings in the city dump. At Twin Falls, Idaho, 125 separate piles were examined in 1945, and in only 2 of these piles were any sclerotia found and there were only a very few in these 2. At Hazelton, Idaho, 75 piles were examined in 1945, and in only 3 were sclerotia found. In 2 of the lots, when sclerotia were found, very few occurred. In 1 lot, however, sclerotia were plentiful and it was possible to secure them by the hundreds from the pile. This lot of seed was traced to a field which had been observed during the growing season. Serious loss from white mold occurred in this field. The grower stated that a large portion of the field was flooded accidentally in the process of irrigation. The field was visited early in October, and it was possible to collect large numbers of sclerotia from the ground. When this field was visited in August the perfect stage of the fungus was found fruiting quite generally in parts of the field which had been flooded.

Life cycle in relation to pathogenicity.—The life cycle of *S. Sclerotiorum* is relatively simple. Initial infection is started by means of the development of apothecia from sclerotia in the soil and subsequent infection of the plants by ascospores. Mycelium in the soil that has remained alive on plant residue and sclerotia may also cause infection. The transmission of the fungus by bean seed has been reported by Baker (2), Blodgett (3), Zaumeyer and Thomas (10), and Doyer (5).

Infection by the organism in the soil.—After the plant has been infected, the spread of the fungus by mycelial growth is very rapid providing climatic conditions are favorable to the fungus. Sclerotia are formed within a few days. These sclerotia may fall to the ground with plant parts and are capable of producing a crop of apothecia with asci and ascospores the same season when weather conditions permit. Or they may lie dormant in the soil until climatical conditions are favorable for their germination.

Experiments were conducted at Moscow, Idaho, to determine to what extent, if any, inoculum from sclerotia and infected plant parts would cause vegetative infection of bean plants and to determine if the fungus is transmitted by infected bean seed.

Pinto U. I. No. 111 beans were used in the experiment. A low percentage of infection was obtained in each case when the following materials were gathered in the field and added to the bean seed when planted 10 days later in sterilized soil in the greenhouse: (a) 1 in. of infested soil from field where disease was present, (b) seed planted adjacent to sclerotia of *S. sclerotiorum* produced on bean plants in the field, (c) seed

[1] Accepted for publication May 26, 1953.
Published with the approval of the Director as Research Paper No. 363 of the Idaho Agricultural Experiment Station.

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planted adjacent to diseased bean stems obtained from the field, (d) seed planted adjacent to decayed bean seed from field where the disease was plentiful.

An experiment was conducted to determine if inoculum from sclerotia of *S. sclerotiorum* and infected bean stems would cause infection of bean plants by vegetative growth of the fungus after the sclerotia and infected bean stems had overwintered in soil at depths of 2-in., 4-in., and 6-in. These materials were buried in screen cages 12 in. square in field soil on the University of Idaho farm at Moscow, Idaho, on October 29, 1949, and were dug up on July 7, 1950. The cages were then buried in soil 1 in. deep and 30 Pinto U. I. No. 111 beans were planted in each cage. The plants were shaded, well watered and grown to maturity. No white mold infection developed at any time on the plants.

INFECTIONS FROM THE SEED.—As a preceding experiment indicated that white mold of beans may be seedborne to some extent, an experiment was conducted using Pinto bean seed from the Hazelton, Idaho, bean dump referred to above. The seed used in this test was somewhat light in weight, and some of the seeds were slightly stained or chalky in appearance. Many sclerotia were present when the seed was gathered.

On November 11, 1949, seeds were planted in 98 pots of sterilized soil using 3 seeds per pot. The final reading was taken on November 25, 1949, when the plants were well emerged. Less than 1 per cent of the plants were infected with *S. Sclerotiorum*.

Cleaned bean seed from fields on 10 different farms in southern Idaho was obtained from the Idaho State Crop Improvement Association. This seed came from bean fields that were badly infected with *S. sclerotiorum* during the 1949 growing season.

Twenty-five slightly shriveled and discolored bean seeds and 25 plump well-colored seeds were selected from each of the 10 lots of seed. Twenty-five slightly shriveled and discolored seed were planted in ½ of a flat of sterilized soil and 25 plump well-colored seeds from the same lot of seed were planted in the other half of the flat. The seeds were planted approximately ¾ in. deep. The flats were observed and notes taken daily. The final reading was taken 17 days later.

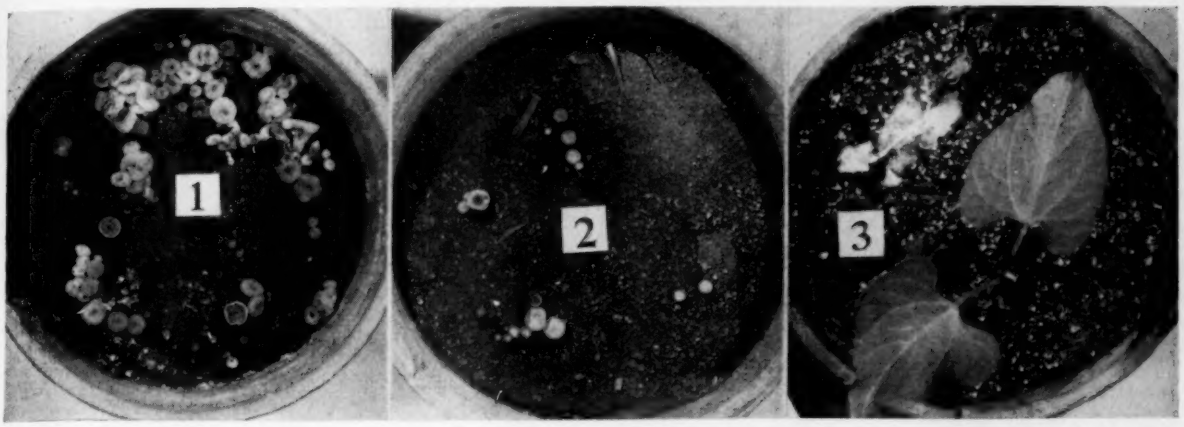
A very small number of plants were infected with white mold. The germination of the seed was poor since only 315 seeds germinated from a total of 500 seeds planted. Fourteen of the 315 plants which grew became infected by *S. sclerotiorum*. Of these 14 infected plants, 11 were from discolored seed and only 3 from plump seed with normal color. Infection appeared in 6 of the 10 lots of seed tested. The possibility exists that some of the seeds were infected with the fungus and the hypocotyl became diseased and died before emerging from the ground. Several of the seeds that failed to emerge were removed from the soil and examined. The seeds were soft and cheesy with no visible sign of white mold infection. The writers were unable to recover *S. sclerotiorum* by plating the rotting seeds on potato agar.

The experiment was repeated after the beans had been stored for 15 months. Of 489 plants grown from 500 seeds planted at this time only 1 plant became infected with white mold.

INFECTION BY THE PERFECT STAGE OF THE FUNGUS.—Coe (4) found that in the greenhouse the behavior of *S. sclerotiorum* in producing apothecia is unpredictable. The writers found this to be true; although little difficulty was experienced in securing germination of sclerotia, it was impossible to predict the length of time taken for germination. The writers used sclerotia produced on bean plants in the field when Coe grew sclerotia from pure cultures. He found that the shortest time necessary for apothecial formation from sclerotia planted in sand to be 18 days and the longest 141 days. The time in this experiment (Table 1) varied from 38 to 162 days when sclerotia were buried 1 in. deep in sterilized soil in pots in the greenhouse. Sclerotia readily germinate, (Table 1), when kept in a dry condition for various lengths of time up to 7

TABLE 1.—*The effect of time of storage of sclerotia of S. sclerotiorum upon its ability to produce apothecium and viable ascospores*

Source of the sclerotia	Treatment	Date of planting sclerotia	Date of production of apothecia	No. of pots planted with sclerotia	No. of pots producing apothecia	Days planting to apothecia
Hazelton bean dump Oct. 1949	Planted 1 in. deep in sterile soil	10-25-49	2-10-50	10	5	162
Hazelton bean dump Oct. 1949	Dried for 2 months at room temperature then planted	12-17-49	1-24-50	15	9	38
Hazelton bean dump Oct. 1949	Stored at room temperature for a period of 1 year then planted	10-6-50	11-20-50	3	3	65
Infested bean fields in 1943	Stored at room temperature for a period of 7 years then planted	10-6-50	11-20-50	3	3	65
Infested bean fields in 1945	Stored at room temperature for a period of 5 years then planted	10-6-50	11-30-50	3	3	75



Figs. 1–3. Fig. 1. Apothecia of *S. sclerotiorum* produced by sclerotia that had been stored in dry packet for 1 year before planting.—Fig. 2. Apothecia of *S. sclerotiorum* produced by sclerotia that had been stored in dry packet for 7 years before planting.—Fig. 3. Seedling Pinto bean plant killed by *S. sclerotiorum*. Infection from ascospores in the greenhouse.

years. More abundant apothecia developed from sclerotia 1 year old, (Fig. 1), than from those 7 years old (Fig. 2).

Sclerotia buried over winter at 2, 4, and 6 in. produced apothecia abundantly in each case when planted in soil 1 in. deep the following summer. Infection was obtained on bean plants grown with the germinating sclerotia under humid conditions.

Natural spread of white mold by ascospores in the greenhouse was demonstrated by the following procedure. Two hundred plants of Pinto U. I. No. 72 were grown in sterilized soil on the center bench of a greenhouse. At 1 end of the greenhouse on a side bench several pots of germinating sclerotia were located. Ascospores discharged from the perithecia in these pots were carried by air currents to the bean plants emerging from the soil. Seventeen of 200 bean plants were infected and killed (Fig. 3). These plants were located from 3 to 13 ft. from the apothecia. Twelve of the plants were infected at the soil line and 5 were infected on the stem near the cotyledons.

After germination the apothecia in several pots were allowed to become dry and in a short time they disintegrated. They were allowed to remain dry from January 9, 1950, until March 1, 1950, when water was added to the soil. By March 17, apothecia were again produced in abundance. Examination of these apothecia revealed the fact that some of them came from sclerotia which had produced apothecia 3 months previously.

SUMMARY

The white mold disease of beans caused by *Sclerotinia sclerotiorum* is considered an important disease in Idaho. Evidence is presented indicating that current season infection by mycelium of the fungus may occur on bean plants from infested soil, from sclerotia, from decayed bean seed and from decayed stems and leaves of bean plants. The fungus was not able to survive the winter at Moscow, Idaho, and cause infection by vegetative growth after overwintering in the soil 2, 4, and 6 in. deep.

Less than 1 per cent seed borne infection resulted when beans from badly infested fields were planted the year following production. Only a trace of the disease was seed borne in 2-year old seed.

Sclerotia held in dry storage for 7 years germinated readily and produced ascospores which caused infection on growing bean plants.

In the greenhouse the ascospores of *S. sclerotiorum* spread readily a distance of 13 ft. to young seedling plants causing infection.

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A BACTERIAL BLIGHT OF CHRYSANTHEMUMS [1]

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During the summer of 1950 our attention was drawn to an instance of rapid and serious loss of florists' chrysanthemums (*Chrysanthemum morifolium* Ram.) in a greenhouse in Buffalo, New York. The necrosis and collapse of the stems, usually first evident in the succulent tissues several inches above the soil line, possessed characteristics unlike those of any well recognized chrysanthemum disease. Bacteria were abundant in the affected tissues and one of those isolated proved to be pathogenic to chrysanthemums, inducing symptoms similar to those expressed in naturally infected plants. Reports of this disease were received in the summers of 1951 and 1952 from Massachusetts and Florida, and specimens from North Carolina, Ohio, Connecticut and Pennsylvania were verified by isolation and inoculation. Losses in most cases were restricted to a few varieties, involving not more than 1–5 per cent of the crop, but in some cases the damage was more extensive, destroying entire beds.

Several bacterial blights of chrysanthemum species have been reported in various parts of the world. Welles and Roldan[2] described a wilt of *C. coronarium* caused by *Pseudomonas solanacearum* in the Philippines. Williams[3] reported a disease of outdoor chrysanthemums in England, the principal symptom of which was drooping of the blooms caused by hollowness of the stem immediately below the flower head. The remaining pith tissue was brown for 2–3 in. The symptoms were reproduced in plants inoculated with a bacterium found to be consistently present in the diseased tissues. Although Williams described the bacterium in some detail, he refrained from giving it a name. Stapp[4] reported a leaf blight of chrysanthemum caused by *Pseudomonas syringae* in Germany.

SYMPTOMS OF BACTERIAL BLIGHT.—Symptoms of the disease in America differ somewhat from those of any of the 3 diseases reported in other countries. Under favorable conditions in commercial greenhouses the first evidence of the blight on established plants is a greyish water-soaked appearance of the stem at a point often over half way from the soil line to the terminal bud. Here, the stem is easily flattened by pressure with the fingers and when cut open longitudinally the pith at the center of the affected area is found to be completely digested, whereas at the upper and lower limits it is reduced to a jelly-like mass. Progress of the disease is then rapid in the succulent tissue of the plant; the area involved completely collapses and the distal portion of the stem falls over (Figs. 1, 2). Internally the diseased stem becomes brown to various shades of red-brown. The diseased area is connected with the base of the plant through brown vascular tissue, necrotic pith, or at times brown diseased streaks in the cortex. If conditions remain favorable rotting may progress downward to the base of the stem, but if at any time conditions become unfavorable further progress of the disease may be checked and axillary buds below the rot may break and give rise to essentially normal shoots (Fig. 2). Although stem rot usually appears first well above the soil line it may in some cases initiate at the base of the stem, producing a rot not easily distinguished from that caused by certain Pythium species. In such cases, the roots also may be extensively rotted.

FIG. 1. Left, 2 Grand Slam plants inoculated near base (at toothpicks); remained essentially symptomless 2 months at low greenhouse humidity; succulent tissues below tip rotted and collapsed 4 days after plants placed under saturated conditions, one plant later wilted. Right, typical stem rot progressing downward after plant decapitated, wound touched with *E. chrysanthemi* suspension, and plant placed at 80°F. and high humidity.

Infected cuttings sometimes are rotted at the base and wilt completely (Fig. 3). Frequently, however, they appear healthy externally, but on examination infection of the internal tissues is revealed. Streaking and discoloration of the vascular tissues and dissolution of the pith is common in such cases. A "hollow stem" disease of cuttings observed by one of the writers prior to 1950 is now believed to have been caused by the bacterium here discussed. It was particularly common on varieties now known to be highly susceptible to bacterial blight.

Symptoms resulting from artificial inoculation agree

[1] Accepted for publication May 27, 1953.

[2] Welles, C. G. and E. F. Roldan. 1923. Another economic host of *Bacterium solanacearum*. Phytopathology 13: 488-491.

[3] Williams, P. H. 1927, 1928. A bacterial disease of chrysanthemum. Ann. Rpt. Exp. and Res. Sta., Turner's Hill, Cheshunt, Herts. 13: 32-38; Ibid. 14: 40-42.

[4] Stapp, C. 1934. Eine Bakteriose an Chrysanthemen. Zentbl. Bakt. etc. Abt. 2. 90: 320-329.

[5] Burkholder, Walter H. and Wilson L. Smith. 1949. *Erwinia atroseptica* (van Hall) Jennison and *Erwinia carotovora* (Jones) Holland. Phytopathology 39: 887-897.

FIG. 2. Naturally infected plant from commercial greenhouse. Presumably the cutting was infected on 1 side when rooted but progress of disease was temporarily checked. Branch at left remained healthy and bloomed normally. First symptoms in stem at right were seen at point where it collapsed; rot progressed downward to point about 2 in. above soil line (arrow) and was then checked. Note healthy new shoot in back which developed from axillary bud just below limit of downward progress of rot.

in nearly all respects with those observed in commercial greenhouses. With somewhat resistant varieties or with highly susceptible varieties when disease conditions are optimum for only a short time, very limited necrotic lesions may develop at the point of inoculation. Watersoaked at first, they dry rapidly and develop a drab brown or red brown color. When the more susceptible varieties are held under favorable conditions following inoculation, progress of the disease is astonishingly rapid. Marked discoloration of the vascular elements is evident 8 in. or more above the point of wound inoculation within 24 hours,

FIG. 3. Upper, bases of Sea Gull cuttings at left dipped in clear water, those at right dipped 1 minute in water suspension of *E. chrysanthemi;* pots placed at 85° F. for 36 hours, then on greenhouse bench. Lower, superficially healthy cuttings split open to show dissolution of pith associated with *E. chrysanthemi* infection.

and within 48 hours wilting of the leaves and dissolution of the pith may be extensive. Often the initial vascular discoloration extends into those leaves whose traces join the infected vascular bundles, and the portions of the laminae directly dependent on those traces wilt irreversibly. This rapid response suggests that a potent toxin is involved, a suggestion supported by frequent failure to recover the pathogen from the distal discolored vascular tissues.

CONDITIONS FAVORING THE DISEASE.—Disease development is highly responsive to conditions of the host and the environment. Succulent growth is most rapidly and severely attacked. Disease development also appears to be dependent upon high temperatures. Symptoms may be very limited or not evident at all at temperatures up to 65°F. However, when inoculated plants of susceptible varieties are exposed to temperatures of 80°F. or higher, pronounced symptoms develop within 24 hours.

Moisture plays an important role in development of the disease, but its effects have not been extensively studied. It has been consistently noted that when infected plants are removed from the moist atmosphere of the infection chamber and returned to the greenhouse where conditions are drier, the progress of disease expression is stopped. This may in part be due to lower average temperature, yet it has been observed in summer when the mean temperature has been well within the range favorable for disease development. Plants inoculated in the greenhouse under dry conditions rarely show evidence of wilting or necrotic lesions. It may be significant also that several serious outbreaks of the disease have occurred during periods of coincidence of high temperature and very high humidity.

Dissemination.—The method of dissemination of the pathogen is not known with certainty. Evidence is convincing, however, that under commercial greenhouse conditions the bacteria may remain relatively inactive in systemically infected stock plants, and cuttings taken from such plants may carry the pathogen to the propagating beds and to other greenhouses. This has been partially substantiated experimentally. The possibility of localized spread on knives or the fingers during the processes of pruning and taking cuttings has been demonstrated (Fig. 1, right).

Varietal susceptibility.—Over 50 varieties of *Chrysanthemum morifolium* have been tested for susceptibility. All appeared to be susceptible when toothpicks previously soaked in a suspension of the bacteria were inserted into the stems, but differences in degree of infection were evident. For example, Mary McArthur, Grand Slam, Forty-Niner, Brocade, and J. W. Prince proved highly susceptible, while Goldsmith, Masterpiece, Mary Lennon Hall, and Blazing Gold were less seriously affected. Some varieties were more affected in the vascular elements, while others showed dissolution of the pith or root rot. These features vary not only among varieties, but even within a variety under different conditions of environment and host development.

Other species of *Chrysanthemum* found to be susceptible to the pathogen are the shasta daisy (*C. maximum* Ramond), feverfew (*C. Parthenium* Pers.), and the Clara Curtis chrysanthemum (*C. rubellum*).

THE PATHOGEN.—The causal organism of the chrysanthemum disease is readily isolated from infected plants, and inoculation experiments with such isolates result in a high percentage of infected individuals. The description of the bacterium that follows is based on 6 such isolates from the chrysanthemum, 3 from naturally infected plants, and 3 from plants artificially infected in the greenhouse. Three isolates from other plants were included in the cultural and biochemical tests since it was thought that they might be identical with the chrysanthemum pathogen. One of these was an isolate (E20) obtained from a rot of celery in 1944 and had been considered an aberrant form of *Erwinia carotovora;* another was isolated by L. Heidrick from a stem rot of *Sedum spectabile* in 1949; a third, obtained from Dr. M. Noble of Edinburgh, Scotland, had been isolated from a potato plant showing the blackleg disease. This later proved to be identical with *Erwinia atroseptica.* Together with the above isolates, cultures of the following were used for comparison: *Erwinia atroseptica, E. carotovora,* and *E. carotovora* f. sp. *parthenii.*

The 6 isolates from chrysanthemum, the 1 from celery, the 1 from *S. spectabile,* and *E. carotovora* f. sp. *parthenii* were identical morphologically, culturally, and physiologically with a few minor exceptions.

They differed in 16 characteristics from *E. atroseptica* and in 10 characteristics from *E. carotovora*. Further work no doubt would demonstrate other peculiarities.

Morphology.—The pathogen is a non-spore-forming straight rod with rounded ends, occurring singly or in pairs. In a 24-hour-old culture on beef-extract-peptone agar, cells varied in size from 1.05μ to 3.85μ in length and .46μ to 1.06μ in width. The mean length was 2.10μ and the mean width .70μ. The pathogen is gram-negative and is motile, with varying numbers of perithrichic flagella.

Cultural characteristics.—On beef-extract-peptone agar (Bacto) slants, growth is moderate, grayish white, and slightly iridescent and butyrous. The medium remains unchanged in color. The celery and the sedum isolates had at times a slight slate gray pigment in the growth. On potato-dextrose agar the growth is more luxuriant, filiform with wavy margin, grayish white to cream, shining and butyrous. Nutrient broth becomes lightly turbid; a light pellicle is formed in about 12–18 hours and soon falls to the bottom. Litmus milk becomes light lavender in 2–3 days with a slight separation of whey. Within a week there is a soft curd formed with ½ the medium whey. The color is pink. Moderate white turbidity is evident in Clara's medium in 24 hours.

Krumwiede's triple sugar agar first becomes yellow in 2 days, then begins to turn red from the top downward until at the end of 2–3 weeks the agar is entirely red. On Endo agar the streak is pink but there is no change in the color of the medium. On desoxycholate agar the growth is yellowish. There is a whitish turbid growth in Fermi's solution.

Physiological studies.—Growth in gelatin is moderate with liquefaction showing on the second day and complete by the tenth. Growth in tryptone broth is good but there was no positive determination for indol with the exception of a slight trace in the sedum culture. A trace of H_2S shows on lead acetate paper suspended over tryptone broth. Nitrates are reduced to nitrites in both synthetic and in beef-extract-peptone broth. The pathogen may utilize nitrates, ammonium salts, and peptone as nitrogen sources. It can utilize asparagine as a combined carbon and nitrogen source. The bacterium is urease negative.

The pathogen, when grown in a synthetic medium (Koser's medium minus the citrate), utilizes the following added carbon compounds: dextrose, galactose, arabinose, xylose, rhamnose, sucrose, dulcitol, glycerol, mannitol, ethyl alcohol (5 per cent), salicin and also the sodium salts of citric, hippuric, malonic and uric acid. Growth in lactose and maltose is slow and acid production is weak. Starch is not hydrolysed but a solidified pectate medium is liquefied.

The pathogen is methyl red negative and acetylmethylcarbinol is formed.

There is only occasional and very slight growth in beef-extract-peptone broth with 5 per cent NaCl, and none above this concentration.

All isolates grow readily at 37°C. Those from chrysanthemums and from guayule grew at 40–41°C. but the isolates from celery and sedum did not. All showed slight growth at 6°C.

The organism is a gas producer. In formate ricinoleate broth, gas appeared in about 3 days and in lactose broth and lauryl sulfate broth in from 1 to 2 weeks. Gas was noted on the third day in Krumwiede's triple sugar agar and occasional bubbles were noted in the pectate medium.

One peculiarity of the species, noted especially in sucrose synthetic broth, was its ability to reduce bromthymol-blue. In 24 hours the indicator was a distinct yellow, but in 72 hours the lower half of the tube was decolorized and in 2 weeks there was a complete decolorization. This reduction was noted in other sugars but was not so striking as in sucrose broth.

Cross inoculation studies.—From the above description of the chrysanthemum pathogen it is readily seen that it belongs in the genus *Erwinia*. This fact led to investigations along 2 lines: first, to determine whether or not it was a soft rot organism; second, to determine whether other related species of *Erwinia* are able to cause a blight of chrysanthemum. The pathogen proved to be as capable of rotting fleshy vegetables as is *E. carotovora*. Inoculation experiments were conducted on the following with positive results: potato tubers, onions, cabbage, lettuce, celery, carrots, and radishes.

Attempts then were made to infect chrysanthemums with other members of the genus *Erwinia*, namely, *E. atroseptica*, *E. carotovora*, and *E. carotovora* f. sp. *parthenii*. The chrysanthemum pathogen was included as a check. Plants of the varieties Mary McArthur and Sea Gull were grown in sterilized soil, and when about 10 in. high, sterilized toothpicks previously soaked in concentrated suspensions of the above bacteria were inserted into the succulent stems. All plants were immediately placed in a moist chamber at a temperature of about 80°F. for 48 hours. This method had proved to be very satisfactory in obtaining infection. Only those plants inoculated with the chrysanthemum organism showed evidence of disease and from them the pathogen could readily be isolated.

Tomato and potato plants also were inoculated with the chrysanthemum pathogen in the manner described above. A complete collapse of the tissue around the point of inoculation resulted and the plant parts above the injury wilted and died.

Taxonomy.—Table 1 shows definitely that the chrysanthemum pathogen in culture is distinct from *Erwinia atroseptica* and *E. carotovora*[5] and pathogenicity tests confirm this point. Cultural characteristics, however, are very similar to those of the guayule pathogen, *E. carotovora* f. sp. *parthenii*, although inoculation tests did not indicate identity of the 2 organisms. Such experiments, however, might not be significant unless conducted with more isolates than were available. Inoculations with the chrysanthemum pathogen were not made on guayule since test plants were not available. A culture of the chrysanthemum pathogen has been sent to Dr. M. P. Starr for comparison with the guayule organism but results have not

TABLE 1.—*Comparison of cultural characters of three species of Erwinia*

Media or tests	*E. atroseptica*	*E. carotovora*	*E. chrysanthemi*
Ethyl alcohol 5%	Not utilized	Utilized	Utilized
Dulcitol 1%	Not utilized	Utilized	Utilized
Sodium malonate .15%	Not utilized	Utilized	Utilized
Sodium hippurate .15%	Not utilized	Utilized	Utilized
Sodium urate .15%	Not utilized	Utilized	Utilized
Sodium tartrate .15%	Not utilized	Utilized	Utilized
Lactose 1%	Rapid acid production	Rapid acid production	Delayed utilization
Krumwiede's triple sugar agar	Medium turns yellow	Medium turns yellow	Medium turns yellow, then red
Brom-thymol-blue in sucrose	Acid. Yellow	Acid. Yellow	Acid. Decolorized
Desoxycholate agar	Pink colonies	Pink colonies	Yellow colonies
Fermi's solution	Growth light to none	Growth light to none	Growth good
Methyl red	Positive	Positive[a]	Negative
Voges-Proskauer	Negative	Negative[a]	Positive
Endo agar	Metallic red colonies Medium deep red	Metallic red colonies Medium deep red	Pink colonies Pink medium
NaCl 5%	Growth	Growth	No growth or trace
Temperature	Max. 32°C.–35°C.	Max. 35°C.–37°C.	Max. 37°C.–41°C.

[a] A large majority of the isolates of *Erwinia carotovora* are M.P. + V.P.—but a few show some variation.

yet been reported. It is felt, however, that the cultural characteristics and the biochemical reactions reveal that the bacterium under consideration is not a *form species* or variety of any known species of *Erwinia*. It therefore should be given specific rank, and the name, *Erwinia chrysanthemi*, is proposed.

SUMMARY

A hitherto unreported disease of greenhouse chrysanthemums was studied. The symptoms are rotting and blighting of the stems of the plants and, at times, of the roots. Lesions are brown to red-brown in color. The disease progresses with great rapidity in succulent tissue under conditions of high temperature and humidity.

The cause of the disease is a bacterial pathogen belonging to the genus *Erwinia*. It has the ability to rot many vegetables but is a species distinct from *E. atroseptica* or *E. carotovora*. The name *Erwinia chrysanthemi* has been given to it.

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SPORE GERMINATION, CULTURAL CHARACTERS, AND CYTOLOGY OF VARIETIES OF USTILAGO STRIIFORMIS AND THE REACTION OF HOSTS [1]

M. J. Thirumalachar and J. G. Dickson

The stripe smuts of the grasses are important factors in the economical production of pasture and forage grasses in the northcentral and northeastern sections of the United States. The 2 species *Ustilago striiformis* (West.) Niessl. and *Urocystis agropyri* (Preuss.) Schroet. comprise the major pathogenes inciting stripe smuts on a similar group of grasses in the area.

In the investigations at Wisconsin emphasis has been placed on the germination of the chlamydospores, cultural characters and cytology of the fungi, and the host reaction of the several grass species. Previous investigators have experienced difficulties or erratic results in securing germination of the chlamydospores and obtaining high infection with these smuts (5, 7, 18, 19, 23, 25, 26, 27, 30). Preliminary studies on these species were reported earlier (28, 29). The present paper represents a summary of the investigations on the varieties and races of *U. striiformis* occurring on 7 grasses of economic importance.

HOSTS, DISTRIBUTION AND IMPORTANCE.—The stripe smut is distributed widely in North America and Europe on many species of grasses, chiefly in the subfamily Festucoideae. While Clinton (6) Zundel and Barnhart (32) record its occurrence in 29 states extending from Maine to California and Minnesota to Texas, apparently the smut is of economic importance chiefly in the more northern states. In Wisconsin the smut is known on 8 hosts: *Agrostis alba* L., *A. palustris Huds.*, *Poa pratensis* L., *P. compressa* L., *Phleum pratense* L., *Dactylis glomerata* L., *Elymus canadensis* L., and *E. virginicus* L. It is extensive on *Bromus inermis* Leys. north and west of Wisconsin to the Pacific Northwest.

The disease is important in the grass land agriculture of the northcentral area. Both yield of forage and seed and permanence of stands are affected by this smut and the similar appearing flag smut. Early pathologists (5,7,25,26) emphasized this fact. Recent investigations (1,21,22,31) have provided more specific data on percentage of infection. Some blue grass pastures and timothy fields in Wisconsin contained 10–25 per cent infection. Most pastures inspected showed a trace to 5 per cent infection of either the stripe smut, flag smut, or more frequently both diseases; yet comprehensive data on the effect of these smuts on yield and plant survival still have not been obtained.

SYMPTOMS.—The symptoms of the stripe smut are similar on the different hosts. The disease first becomes manifest by the development of long, narrow sori on the leaves and leaf sheaths. The sori in the first few leaves developing are small and sparsely distributed. Lesions are abundant and coalesce to form elongated stripes and shredding of leaves in the foliage developing later. The conspicuous smut early in the spring is the flag smut and the stripe smut becomes the most conspicuous in late May and June in Wisconsin. Internodal elongation is reduced in infected plants and usually the inflorescence primordia are replaced by strands of sori. A few infected plants in red top, timothy, and blue grass develop culms and inflorescences with sori evident in culms, rachises, and spikelets. Smutted plants usually do not live beyond the second year after infection under Wisconsin conditions.

PATHOLOGICAL HISTOLOGY.—Smutted leaves of red top, blue grass, timothy, and orchard grass showed a similar pathological histology. Dicaryotic mycelium, lobed and much branched, developed between the cells of the mesophyll tissue. The mesophyll cells were compressed and disorganized during the early stages of spore formation, with the sorus occupying fully the interveinal mesophyll between the upper and lower epidermal layers. Mycelium was abundant along the adjoining vascular bundles and frequently penetrated into the xylem vessels although the vascular tissues were not disorganized. As mature spores accumulated the epidermal layers were ruptured over the sorus and the spores were liberated. Following spore dispersal the leaves shredded and the leaf tissues bleached and dried. Sori developed abundantly in the partially differentiated meristem of culm and inflorescence with only the early vascular bundles persisting.

Mycelium and spores were present in the floral bracts and chalazal tissue of the caryopses on less severely damaged plants. Fully developed caryopses were not found in the material studied. The seed-borne nature of the fungus was first suggested by Osner (25) who found the mycelium within the tissues of the ovary. Kreitlow (20) suggested seed-borne infection in germination studies with timothy.

Perennial mycelium of the fungus was demonstrated in the crown of timothy plants. Mycelium was present in the parenchyma and around the xylem in sections of the enlarged basal culm tissues of timothy plants collected in autumn and spring. The hyphae were both inter- and intracellular in the parenchymatous tissues. As in the leaf tissue, hyphae were established in close contact with the xylem vessels with branches penetrating the xylem cells.

Penetration and establishment of the fungus in the grass seedlings were investigated with susceptible grass hosts. High percentages of infection were ob-

[1] Accepted for publication June 9, 1953.
Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. The illustrations were prepared by E. H. Herrling, Department of Plant Pathology, University of Wisconsin.

tained by germinating the grass seedlings on moistened filter paper in contact with the germinable spores, dicaryotic mycelium, or compatible sporidia. The dishes with seed and inoculum were inverted until the young coleoptiles of the seedlings were 5–10 mm. long. This insured continuous contact between the coleoptiles and inoculum.

In the susceptible host the infection hyphae entered the host epidermal cells, or between these by direct penetration through the cuticle of the young coleoptile (Pl. II, 1 A-C and Pl. I D and G). Generally the chlamydospores germinated by forming the promycelium and direct conjugation of the compatible cells (Pl. I A and B). Frequently the conjugation enlargement of the promycelium functioned as an appressorium-like structure and the fine infection hyphae penetrated the cuticle beneath this swollen portion of the promycelium. The advance of the mycelium in the coleoptile was intracellular more commonly than intercellular. The nuclei of the invaded cells were orientated near the point of penetration (Pl. II, 1 B and Pl. I G). The hyphae enlarged and branched in the advance through the parenchymatous tissues towards the crown primordia (Pl. I H). Sinuate, tortuous hyphae developed along the walls of the xylem and phloem in the advance through the subcrown internode (Pl. II, 1 J). Frequently the cells of the phloem parenchyma were penetrated, and lobed to irregular shaped hyphae filled the cell lumen. Establishment of the mycelium in the crown primordium was similar to that in the parenchyma, with the hyphae advancing into the differentiating tissues of the culm apex. Penetration of leaf tissue was common in the mycelial advance through the young seedling.

The pathological histology of the fungus from red top in seedlings of blue grass and timothy was different from that in the compatible hosts described above In blue grass infection hyphae developed in abundance in close contact with the cuticle over the epidermis of the coleoptile and the subcrown internode. A low percentage of penetration through the cuticle between or into the epidermal cells occurred in the blue grass seedlings. The hyphae developed into thickened knobs or short, thickened branches. The host nuclei in the invaded cells were oriented near the place of penetration as in the penetration of the susceptible host tissue. In the blue grass seedlings, the protoplasm of the invaded cells and later those adjacent to the infection hyphae showed a deep staining reaction with acid fuchsin and finally general collapse of the protoplasm occurred (Pl. I E). The infection hyphae developed little beyond the initial penetration. In cleared, unstained seedlings the invasion points were evident by necrosis and orange-yellow flecks. The reaction of the tissues in the blue grass seedlings was similar to that described by Evans (11) in maturing onion tissues invaded by *Urocystis cepulae* Frost. Seedlings of timothy inoculated with spores from red top again showed abundant infection hyphae over the epidermis. Penetration through the cuticle into or between the epidermal cells of the coleoptile or subcrown internode occurred without marked evidence of host reaction (Pl. II, 1 D, F, and G). However, the mycelium in the epidermal cells and adjacent mesophyll or cortical cells developed thin, tapering hyphae with reduced protoplasmic contents chiefly at the apex (Pl. II, 1 H and Pl. I, F). Further development of the pathogen toward the culm primordia was limited.

The Fungus

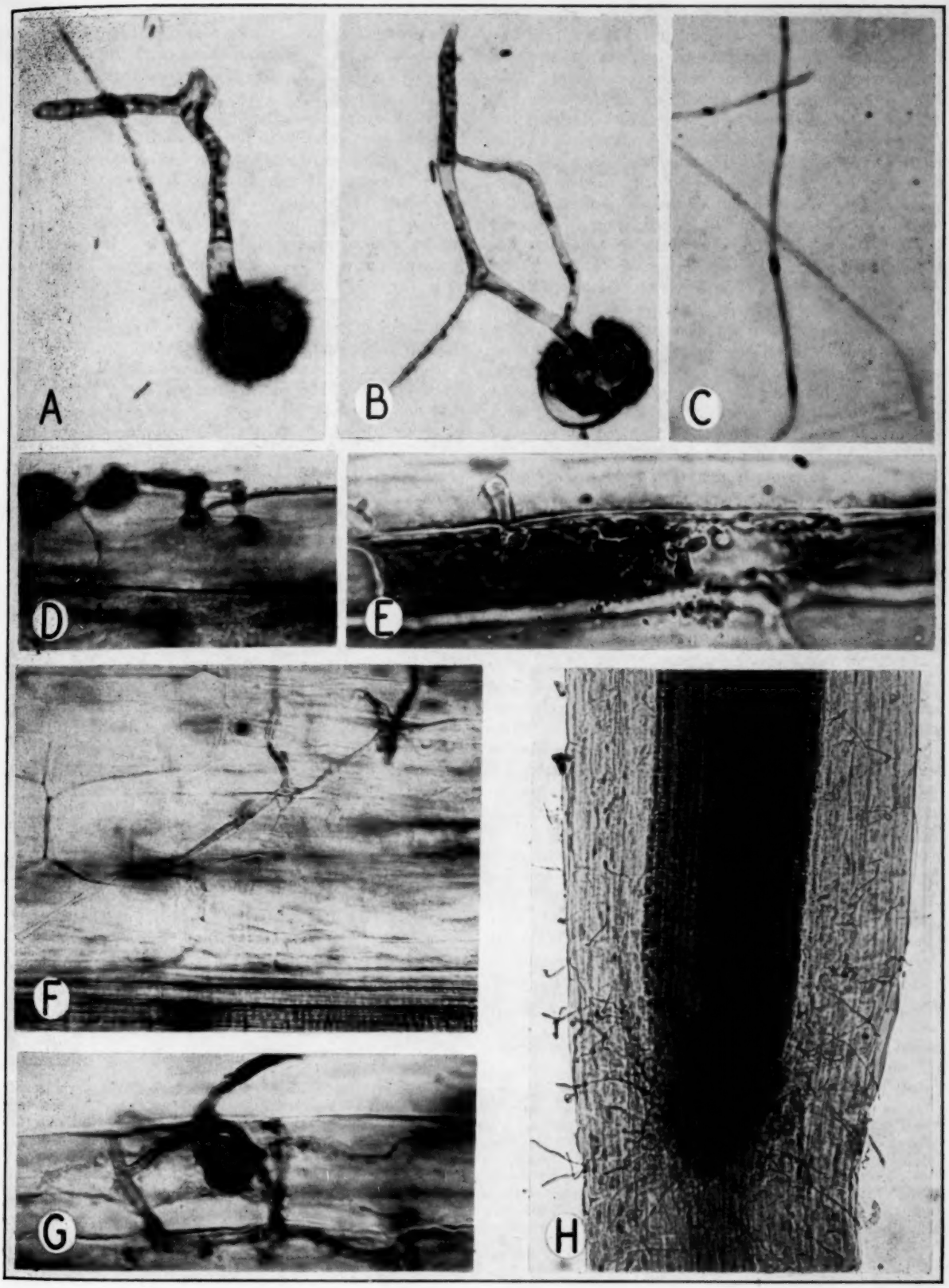
Ustilago striiformis (Westend.) Niessl, Hedwigia 15:1. 1876. Syn. *U. striaeformis* (Westend.) Niessl

Sori are formed in leaves, leaf sheaths, and primordia of inflorescences. They are linear, dark brown to almost black, powdery, and give leaves a shredded appearance after dispersal of spores. Chlamydospores are chiefly globose to subglobose or ellipsoid, dark brown, prominently echinulate, and chiefly 9–12 microns in diameter. Spore germination is chiefly of 3 types: 1) the formation of a promycelium without sporidia and conjugations between compatible cells of the promycelium (Pl. I, A and B, Pl. II); 2) the development of a promycelium bearing ellipsoid sporidia (Pl. II, 3 J and K); 3) the growth of an irregular promycelium with lateral branches sometimes bearing sporidia and conjugation between compatible sporidia or monocaryotic cells of the branches (Pl. II, 3 A-H and 4 A-G). Germination is frequently erratic and spores frequently require after-ripening or special treatment to counteract an inhibitor.[2] The species shows great variation in cultural characteristics and nuclear condition on media.

Specialized varieties and physiologic races.—*On Gramineae: general in distribution.*—1) *Basis for varieties and races.*—The history and the classification of the pathogen were reviewed by Osner (25) and Fischer (12, 14). The similarity of the symptoms on the various hosts and the close resemblance in size and shape of chlamydospores have led most mycologists to apply the name *U. striiformis* for the stripe smut on some 75 hosts (8, 12, 14). Liro (24), Ciferri (4) and Gutner (16) have differentiated several species on

[2] The present investigations indicate an inhibitor generally present in limited amounts in the freshly matured spores under the host epidermis and increasing in amounts as the spores are released.

Plate I. Spore germination, host penetration, and development of mycelium of *Ustilago striiformis* var. *agrostidis*. A) and B) Characteristic spore germination and initiation of the dicaryotic mycelium. ×1000. C) Dicaryotic mycelium from culture showing the close association of nuclei. Spores of same culture are shown in Plate IV, G. ×1000. D) Two direct penetrations into an epidermal cell of the coleoptile of red top seedling. ×900. E) Typical incompatible reaction of cell of coleoptile of blue grass when invaded by var. *agrostidis*. ×1000. F) Penetration and mycelial development through the cells of the coleoptile of timothy showing the characteristic depleted hyphae. ×900. G) Penetration and development of hyphae in the coleoptile of red top, a compatible reaction between host and pathogen. ×1000. H) Showing mycelium of var. *agrostidis* in coleoptile, subcrown internode, and primordium of red top. ×100.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the basis of minor morphological differences in spore size and character or pathogenicity. Fischer (13, 14) has summarized the literature on the nomenclature and presented sound argument for the permanent establishment of the collective species under *U. striiformis*.

The differentiation of the specialization within the collective species *U. striiformis* is presented on the basis of specialized varieties and physiologic races as suggested by Fischer and Shaw (15). Earlier reports pertaining to specialization of the stripe smut on the several hosts included in this investigation are covered briefly. Davis (8, 9), on the basis of cross inoculations, listed the following forms of *U. striiformis* (*striaeformis*): *forma Phlei* Davis on *Phleum pratense* L.; *f. Agrostidis* Davis on *Agrostis palustris* Huds.; *f. Poae-pratensis* Davis on *Poa pratensis* L.; and *f. Poae-annuae* Davis on *P. annua L.* He placed the smut on orchard grass in a new species, *U. clintoniana* Davis, based on slight differences in spore size and color in mass. Ciferri (4) in a restudy of the early stripe smut collections made by Davis at Madison, Wisconsin, raised certain physiologic forms to specific rank as follows: *U. phlei-pratensis* Davis and *U. agrostis-palustris* Davis, thereby assigning the new species to Davis. Davis (9), however, retained the physiologic forms originally proposed. Fischer (12) described the stripe smut on *Elymus* and *Agropyron* spp. in the northwestern United States as germinating with the formation of sporidia and well developed sexuality. Following cross inoculations he designated it as *U. striiformis f. hordei* Fischer.

Although aware that no useful purpose is served by unnecessary changes, the writers feel that sufficient study of chlamydospore germination, cultural types and cross inoculations have been made to demonstrate the need for systematic subdivisions of the species on a trinomial basis. The parallelism in the types of germination, range in cultural characters or types, instability or heterogeneity of the cultures and cross inoculations with 6 isolates from different hosts justify the retention of the collective species, *U. striiformis*. Varieties are used to designate certain minor morphological variations and especially host specificity within genera of the Gramineae. Physiologic races are then designated by number under each variety. Wide differences in the morphology of spore germination occur; ranging from the development of promycelia without sporidia and conjugation (Pl. II, 2) between the cells of the promycelia as in *U. nuda* (Jens.) Rostr. in some isolates of *U. striiformis* from *Agrostis* and *Bromus* to typical sporidial production in most isolates from *Hordeum* and *Elymus* (Pl. II, 3). Spore germination and mycelial development indicate a variable species with wide differences in morphology appearing frequently.

The following changes in nomenclature are proposed based on the life cycle in the host and *in vitro*.

a). *Ustilago striiformis* (Westend.) Niessl var. *agrostidis* (Davis) n. comb. Syn. *U. striaeformis* (Westend.) Niessl *f. agrostidis* Davis.

Germination of the chlamydospores is by promycelium with conjugation between cells or branched promycelium with conjugation between cells, generally without sporidial formation on the promycelium (Pl. I, A and B, Pl. II, 2). Mycelial and sporidial growth both occur in culture (Pl. III, C and D).

On *Agrostis alba* L., *A. palustris* Huds.

b) *Ustilago striiformis* (Westend.) Niessl var. *dactylidis* nom. nov. Syn. *U. clintoniana* Davis.

Chlamydospores are slightly larger or the same as those on other hosts. Germination of the spores is by promycelium of upturned, incurved, or helicoid branches (Pl. II, 3 A-H) with conjugation between cells of branches, generally without sporidial formation. Mycelial type of growth in culture.

On *Dactylis glomerata* L.

c) *Ustilago striiformis* (Westend.) Niessl var. *hordei* G. W. Fischer Syn. *U. striiformis* (Westend.) Niessl *f. hordei* G. W. Fischer.

Germination of chlamydospores is by 1 to several strong promycelia developing sporidia (Pl. II, 3 J and K). Detached sporidia conjugate and produce infection hyphae (Pl. II, 3 L and M). Sporidial and mycelial type of growth develop in culture.

On *Elymus*, *Agropyron*, and *Hordeum* species.

d) *Ustilago striiformis* (Westend.) Niessl var. *phlei* n. comb. Syn. *U. striaeformis* (Westend.) Niessl *f. Phlei* Davis.

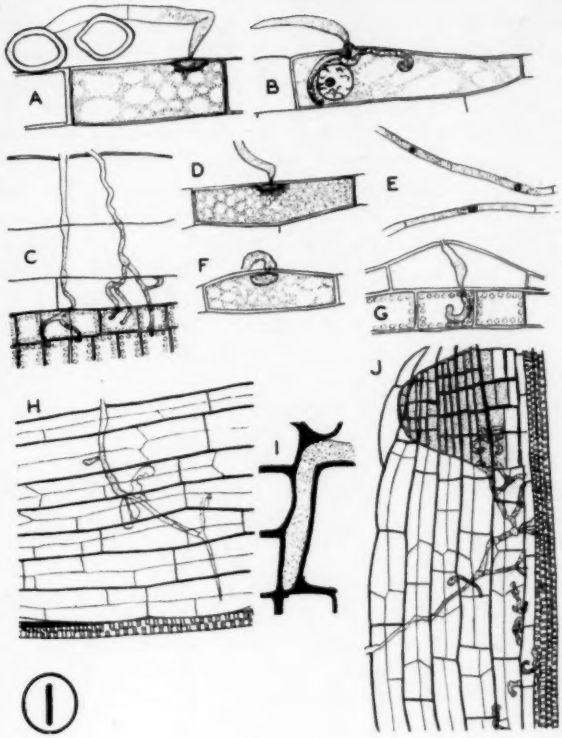
Germination of the chlamydospores is by branched promycelium frequently with sporidia abstricted. Mycelial and sporidial type of growth are common in culture (Pl. III, A-E).

On *Phleum pratense* L.

e) *Ustilago striiformis* (Westend.) Niessl var. *poae* nom. nov. Syn. *U. striaeformis* (Westend.) Niessl *f. poae-pratensis* Davis.[3]

[3] According to Article 28 of the International Rules of Botanical Nomenclature, the use of binary nomenclature for subdivisions of species is not admissible and it is recommended to reduce them to ternary names.

Plate II. Drawings showing host penetration, spore germination, and initiation of the dicaryophase in varieties of *Ustilago striiformis*. 1) Penetration and infection by *Ustilago striiformis* var. *agrostidis* in grass seedlings. A) B) and C) Penetration into the coleoptile of red top (*Agrostis alba*) seedlings. ×900. D) F) G) and H) Infection hyphae in the coleoptile of timothy (*Phleum pratensis*) ×450. I) Mycelium between cells in the coleoptile of red top. ×1000. J) Mycelium in the subcrown internode of red top. ×400. 2) Spore germination and cell conjugation in isolates of *Ustilago striiformis* var. *agrostidis*. A–D) Young promycelia showing number and location of nuclei prior to conjugation. ×450. E–L) Type compatibility and conjugation between cells of the promycelia. ×450. 3) Spore germination, cell conjugation and chlamydospores of *Ustilago striiformis*. A–H) Spore germination and nuclear orientation in var. *dactylidis*. ×450. F) Monocaryotic and G) Dicaryotic mycelium from cultures. ×900. I) Shape of spores produced in culture. J) K) Sporidial production characteristic of var. *hordei*. ×450. L–N) Sporidial conjugation in var. *hordei*. ×450. 4) Spore germination and chlamydospore development in culture of *Ustilago striiformis* var. *poae*. A–E) Promycelial development and sparse sporidial formation. ×450. F) G) Secondary sporidial formation. ×900. H–M) Range in type of spore-like cells in culture. ×450. N) Germination of spore from culture. ×450.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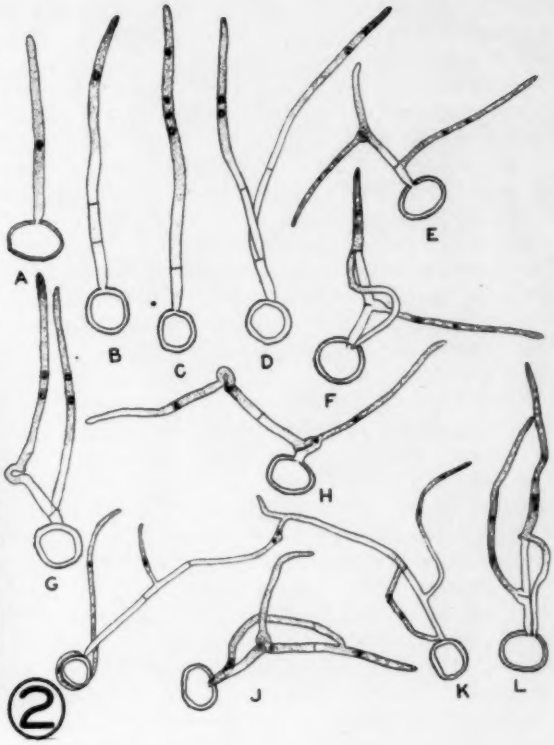
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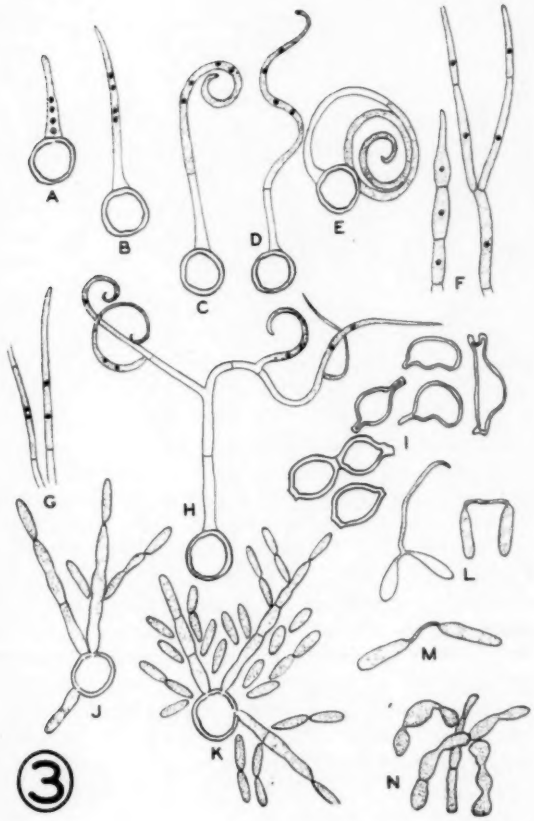
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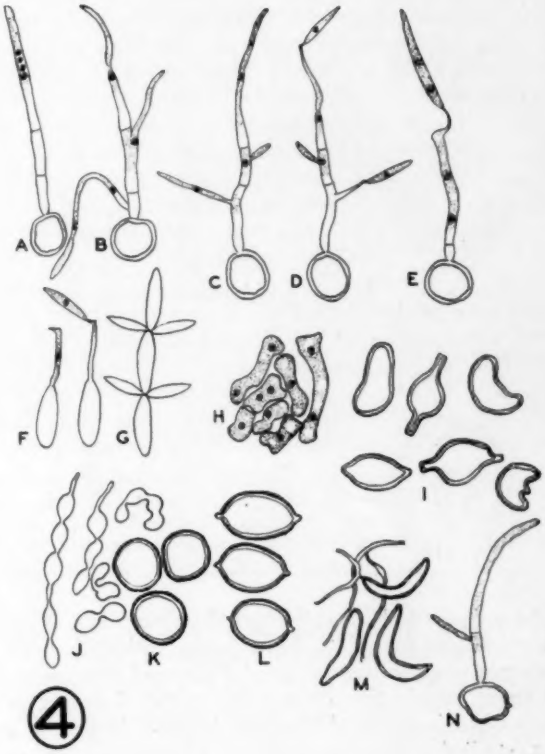
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Germination of spores and growth in culture are similar to *U. striiformis* var. *phlei* in the cultures studied (Pl. II, 4 A-G).

On *Poa pratensis* L., *P. compressa* L., *P. annua* L.

Physiologic race P-1 from *P. pratensis* did not infect *P. compressa* and C-1 from *P. compressa* did not infect *P. pratensis* with the Wisconsin collections studied and according to Davis (9). A tentative third race, A-1 on *P. annua* is set up on the basis of Davis' investigations.

Inoculation studies with pure lines and clonal lines of some of the grass hosts mentioned indicated races within the species. Sufficient cross inoculations have not been made to designate races beyond those indicated above.

2) *Physiology, cytology, and pathology of varieties of the fungus.*—Comparative investigations were made on several isolates of the major varieties of the pathogen. Spore germination, cultural characteristics of mono- and dicaryotic mycelium, nuclear cycle and pathogenicity were investigated especially in *agrostidis, poae, phlei,* and *dactylidis.*

Germination of chlamydospores was erratic at times in all varieties of the fungus. After-ripening of the spores has been considered by previous investigators to be an important factor in erratic germination. The presence of an inhibitor was indicated by these investigations. Good germination of the spores generally occurred on open slides over a free water surface, in the presence of an adsorbing agent, and in dilute solutions of casein hydrolysate, naphthoquinones, etc. However, spores removed from the smutted plants at different seasons of the year and locations in the sori responded differently to germination and to treatments. Season of the year, vegetative condition of the host and temperature appeared to influence the germinability of the spores in water on the open slides or in 3 per cent solution of casein hydrolysate (Table 1).

TABLE 1.—*Average percentage germination of chlamydospores collected from plants, May through November at Madison, Wisconsin*

Date collected and germinated	Percentage of germination of spores of: *poae*	*phlei*
May 4	6	8
May 20	8	8
June 1	10	10
June 18	15	18
July 20	80	75
July 22	75	70
July 24	82	76
Aug. 20	25	20
Sept. 15	20	10
Oct. 10	15	—
Nov. 28	3	2

At some periods of collection the percentage of viable spores appeared to be a factor rather than the presence of an inhibitor. The influence of temperature on spore germination on samples collected during periods of high germinability was different in the varieties studied. Low percentages of germination occurred at 4 and 8°C. in spores of varieties *agrostidis, poae,* and *phlei.* Germination above 70 per cent occurred in *agrostidis* from 20 to 32°C., in *poae* from 28 to 32°C. in *phlei* only at 32°C. At the low temperatures, the promycelia were slender, frequently branched, and conjugations were less common. At high temperatures, the promycelia were short, septate and frequently fragmented into sporidia-like structures. Conjugations were less evident at high temperatures. Some isolates of varieties *phlei* and *poae* produced antibiotics highly effective in inhibiting the growth of 4 filamentous fungi (Pl. III, F).

Both mono- and dicaryotic mycelial cultures were obtained from all varieties studied. Sporidial cultures were common in *hordei,* less common in *poae* and *phlei* and uncommon in *agrostidis* and *dactylidis,* although sporidia-like sectors occurred from the mycelium of these latter varieties. Mycelial types in culture were somewhat similar in range over the groups of varieties studied. Dicaryotic mycelial growth of the cultures studied showed a difference in temperature response. Mycelial types of *phlei* and *poae* developed at higher temperatures than *agrostidis* (Pl. IV). The great diversity in cultural characteristics and variation in nuclear condition illustrated the extreme variability found in many of the Ustilaginaceae. The change from dicaryotic to monocaryotic condition in sectors of the mycelial colonies apparently was associated with the development of mycelium or sporidia from uninucleate cells. Sectors of this type were observed in all cultures studied. These illustrate perhaps Buller's (3) suggested process of dediploidization and were essentially similar to the condition described by Bauch (2), Dickinson (10), Hanna (17), and others in several species of Ustilago. Initiation of the dicaryophase also showed considerable variation. Characteristically, following the reduction divisions in the promycelium, conjugation occurred between compatible cells of the promycelium (in *agrostidis*) (Pl. II, 2) or between compatible sporidia (in *hordei*). (Pl. II, 3 L and M). In the other varieties studied conjugation occurred between compatible cells in branches of the promycelium or in mycelium in culture. Not infrequently nuclei paired in branches of the promycelium before septation to give rise to the dicaryophase (Pl. II, 3 H). Fusion of these nuclei in the mycelium to produce the syncaryotic nuclei as described by Leach and Ryan (23) was not observed although searched for diligently. Frequently paired nuclei of the dicaryophase were so closely associated that in slightly over stained preparations they appeared as a single lobed or ovate nucleus (Pl. I, C). However, the appearance of the dicaryotic condition in the young hyphae of these cultures was in marked contrast to the dicaryotic and diploid condition in the mature chlamydospores developing in the cultures (Pl. III, G). The formation of the diploid chlamydospores was observed in some isolates of 4 varieties (Pl. II, 4 and Pl. III) and spore-like cells were formed in the mycelial culture of the fifth, *hordei* (Pl. II, 3 I). The germination of the chlamydospores formed in culture, however, was not wholly comparable to that of those produced in the

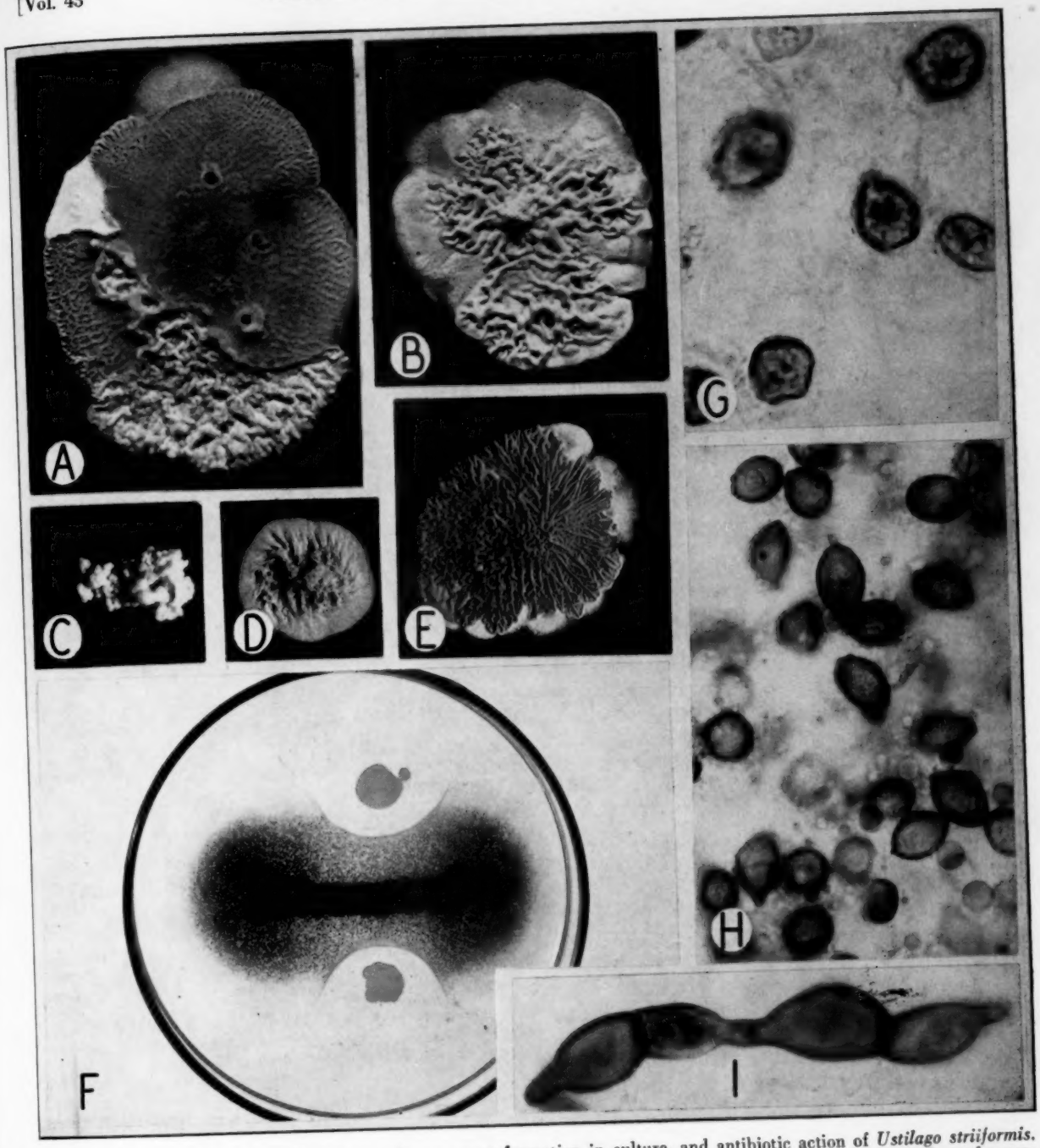

Plate III. Mycelial and sporidial type cultures, spore formation in culture, and antibiotic action of *Ustilago striiformis*. A) Sectoring colony of var. *Phlei*. B) and E) Two different types of colonies of var. *phlei*. C) and D) Same for var. *agrostidis*. F) Showing antibiotic action of two sporidial colonies of var. *poae* to *Aspergillus niger*. G) Typical dicaryotic and diploid spores of var. *agrostidis* that germinate with a characteristic promycelium. ×900. H) Typical spores produced in a culture of var. *poae*. ×900. I) Dicaryotic, spore-like cells in a culture of var. *phleum*. ×900.

host as the branched promycelium was not distinctive in type as were those from the original spores in certain varieties (Pl. II, 4 N).

Cross inoculations between varieties of the pathogen were made with seedlings and crown buds on older plants. Germination of seedlings in the presence of the spores between moist filter papers in inverted petri dishes until the coleoptile had elongated about 1 cm. and planting in soil resulted in smut percentages ranging between 45 and 90 per cent in the susceptible hosts. Infection was determined also in the seedlings following fixation, clearing, staining, and mounting the whole

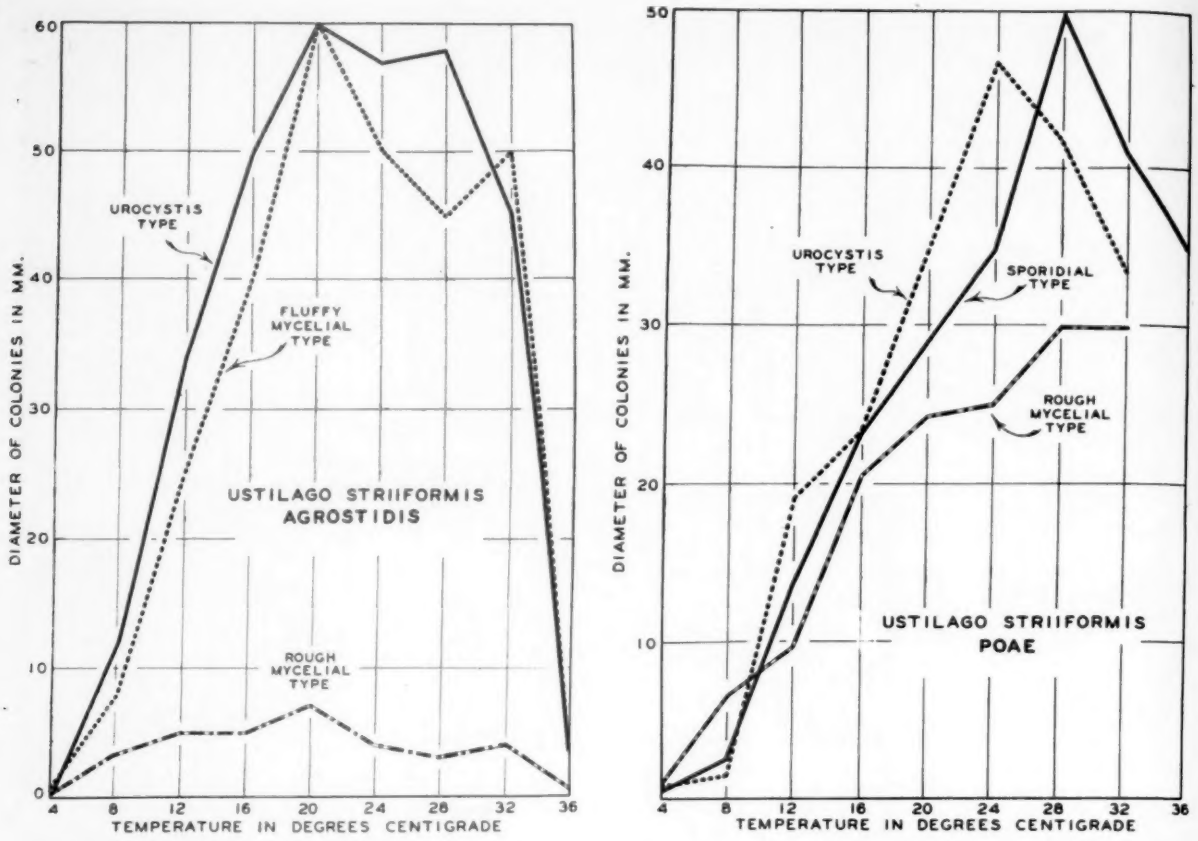

Plate IV. Graphs showing the influence of temperature on the radial growth of three cultural types each of *Ustilago striiformis* var. *agrostidis* and var. *poae*.

seedling. Inoculating crown buds by decapitating the enclosing leaf whorl or by hypodermic injection of the inoculum resulted in differential infection data. Infections were obtained using spores, mixed sporidia, and dicaryotic mycelium. No infection was obtained with sporidial lines or monocaryotic mycelium of the several varieties studied.

SUMMARY

Cultural characteristics and cross inoculations were used to differentiate 5 varieties of *U. striiformis* inciting the stripe smut of grasses. Trinomials are suggested for this pathogen occuring on a large number of grasses.

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A PHYTOPATHOLOGICAL NOTE

The Role of Pectic Enzymes in Fusarium Wilt of Tomato.[1] S. S. Gothoskar, R. P. Scheffer, J. C. Walker, and M. A. Stahmann. The systemic toxic theory of wilting in the vascular fusarial diseases of plants has been supported by Gäumann and co-workers[2] who identified a polypeptide, lycomarasmin, and proposed it as the chief cause of wilting of tomato infected with *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen. Scheffer and Walker,[3] in presenting evidence against lycomarasmin as the chief wilting toxin, reported a thermolabile, non-dialyzable factor in replacement culture filtrates which caused vascular browning and wilt typical of diseased tomato plants. The present paper is a preliminary report of further work on the indentification of the browning and wilting factor produced by the tomato wilt organism. A more extensive paper will be published later.

Optimum culture conditions for production of the wilt and vascular browning factor were determined first. Filtrates or culture extracts were assayed by a standard technique, in which a series of dilutions was used and tomato cuttings were allowed to take up the fluid with the transpiration stream. Four days after treatment the cuttings were graded for degree of wilt and vascular browning. Classes were designated according to increasing degrees of wilt from 0 to 4. The degree of browning was rated in 5 classes as follows: 0 = no browning; 1 = slight browning in lower portion of stem; 2 = browning in lower 1/3 of stem; 3 = browning in the lower 2/3 of stem; 4 = browning of all bundles from base to tip of stem. Several different cultural techniques were used, including ordinary cultures with various carbon sources and various levels of glucose, replacement cultures, and bran cultures. The most active preparation was obtained by growing the fungus on sterile moist wheat bran for 3–6 days and extracting the cultures with water.

Since the non-dialyzable, thermolabile nature of the active substance suggested that a protein might be involved in the development of symptoms, bran culture extracts were examined for their enzymatic activities. Commercial preparations of papain, pepsin, trypsin, erepsin, diastase, alpha-amylase, beta-amylase, beta-glucuronidase, lipase, emulsin, hemicellulase and pectinase were also tested on tomato cuttings. Only those preparations containing pectic enzyme activity produced typical disease symptoms. Some of the others caused wilt but none caused vascular browning. Examination of the bran culture extracts showed that they contained a significant amount of pectin methyl esterase (PME) activity. The extract was purified by precipitation with alcohol (66 per cent final concentration) to give a fluffy powder which had good PME activity but very low polygalacturanase (PG) activity (Table 1). When tomato cuttings were treated with

[1] This investigation, conducted in the Departments of Biochemistry and Plant Pathology, University of Wisconsin, was supported in part by grants from the Research Committee of the Graduate School, and from the Herman Frasch Foundation.

[2] Gäumann, E. 1951. Some problems of pathological wilting in plants. Advances in Enzymology 11: 401-431.

[3] Scheffer, R. P. and J. C. Walker. 1953. The physiology of Fusarium wilt of tomato. Phytopathology 43: 116-125.

TABLE 1.—*Enzyme activity of and reaction of tomato cuttings to several pectic enzyme preparations*

Enzyme preparation and source	Enzyme activity		Reaction of cuttings in concentration of preparation indicated			
			Wilt [b]		Vascular browning [b]	
	PME [a]	PG [a]	0.25 per cent	0.5 per cent	0.25 per cent	0.5 per cent
Pectinol 100D (Rohm & Haas)	272.4	20.0	3.3	4.0	3.0	3.3
Pectin methyl esterase (Takamine Lab. Inc.)	255.4	22.4	3.5	3.5	2.3	3.3
Pectinase (Nutritional Biochemical Corp.)	171.1	12.8			2.0	2.0
Hemicellulase (Nutritional Biochemical Corp.)	78.2	0.6	3.0	3.0	1.0	2.3
Pectinol M 129B (Rohm & Haas)	34.3	1.7	1.7	2.5	1.0	1.0
Pectinol M 137B (Rohm & Haas)	17.2	0.6	1.5	2.3	0.3	1.0
Preparation from the tomato wilt fungus	88.7	0.2	2.5	3.0	2.3	2.7

[a] See text for explanation of values.
[b] Average values of 4 cuttings each. See text for explanation of values.

dilute solutions of this preparation, typical disease symptoms were produced, including decreased transpiration, vessel plugging, vascular browning, and wilt.

To test further the hypothesis that pectic enzymes could reproduce disease symptoms, pectic enzymes from several sources were used (Table 1). These preparations were assayed for PME by measuring the mg. of methoxyl groups hydrolyzed from 1 per cent pectin solution by 1 gm. of enzyme preparation in 3 hours. PG activity was expressed in units per gram of preparation, a unit being the amount of enzyme required to liberate 0.05 milliequivalent reducing groups in 5 minutes in a 5 ml. aliquot from a 100 ml. reaction mixture of 0.5 per cent sodium polypectate. Tomato cuttings were allowed to take up dilute enzyme solutions with the transpiration stream and graded later for symptoms as described above. A correlation between symptoms produced, especially vascular browning, and PME activity was obtained with 7 different preparations (Table 1). There was no consistent correlation of vascular browning with PG activity. Preparations with good PME activity but entirely free of PG have not been obtained to date.

The mechanism of the action of PME in bringing about wilt and vascular browning has not been fully established. However, it appears that cellular organization is disrupted in some way to release a viscous material into the xylem vessels so that plugging occurs, thus causing wilt.[3] Phenol oxidases and melanin formation are probably involved in the browning reaction. This is indicated by the fact that very good browning was obtained by introducing dilute solutions of various phenolic compounds into healthy cuttings. Hydroquinone or catechol, for example, in 0.005 per cent solution caused striking vascular discoloration but no wilting.

From these experiments it is evident that *F. oxysporum* f. *lycopersici* produces a significant quantity of extracellular pectic enzymes which consist of a large proportion of PME (pectin methyl esterase) and relatively little PG (polygalacturanase). Since the enzyme preparation produces the typical symptoms of tomato wilt it is postulated that PME is the most imporant single factor in causing the wilt and vascular discoloration characteristic of infected tomato plants. The initial establishment of the fungus as a successful parasite in the susceptible tomato may well depend on other properties of the organism.—University of Wisconsin, Madison 6, Wisconsin.